Ataxin-1 in cognition and mood

A THESIS

SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL

OF THE UNIVERSITY OF MINNESOTA

BY

Melissa Jean Asher

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

Marija Cvetanovic, Ph.D.

November 2017

Copyright 2017 Melissa Jean Asher

Dedicated to the memory of my uncle Terry, who inspired a love of science in everyone he met.

Abstract

Ataxin-1 *(ATXN1)*, the gene mutated in spinocerebellar ataxia type 1 (SCA1), may affect cognition and mood, but much remains unknown, including which brain areas are responsible, whether *ATXN1* affects mood in mice, and the mechanisms of these effects. To answer these questions, we characterized cognition and mood in several *ATXN1* mutant mouse lines: *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice to compare 50% and 100% loss of *ATXN1*; *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ mice to compare SCA1-like polyglutamine expansions of different lengths; and Purkinje cell specific *Pcp2-ATXN1[82Q]* mice to determine the cerebellar contribution. *Atxn1*$^{-/-}$ and *Atxn1*$^{154Q/2Q}$ mice showed cognitive deficits. Reduced hippocampal neurogenesis in *Atxn1*$^{-/-}$ mice may explain this. Cognition was not affected in *Atxn1*$^{+/-}$, *Atxn1*$^{78Q/2Q}$, or *Pcp2-ATXN1[82Q]* mice. We also observed mood abnormalities *not* consistent with depression or anxiety. These results provide a foundation for further research into the function of ataxin-1 and the potential side effects of reducing ataxin-1 levels to treat SCA1.

Table of Contents

List of tables

Discussion

List of figures

Introduction

Ataxin-1 (ATXN1), located on the short arm of chromosome 6, is a highly evolutionarily conserved gene which is expressed widely throughout the brain (Banfi *et al.* 1996). *ATXN1* encodes the protein ataxin-1 which is thought to contribute to both transcriptional regulation and RNA splicing by interacting with transcriptional repressors, such as *Capicua* (CIC) (Lam *et al*. 2006) and components of the spliceosome, such as RBM17 (Lim *et al*. 2008), affecting their activity. This means it is poised to affect a wide variety of downstream cellular processes.

The human *ATXN1* gene contains an unstable CAG tract, with the number of CAG repeats varying widely between individuals. Healthy individuals have between 6 and 44 CAG repeats (Quan, Janas, and Popovich 1995; Matilla *et al.* 1993), with those above 38 repeats having one or more CAT interruptions. The CAG tract is translated into a polyglutamine tract in the ataxin-1 protein. Long uninterrupted polyglutamine tracts (more than 39 residues with no histidine interruptions from CAT codons) affect ataxin-1's aggregation (Watase *et al*. 2002), degradation (H.K. Chen *et al*. 2003), and interactions with its binding partners (Lim *et al.* 2008), leading to the fatal neurodegenerative disease spinocerebellar ataxia type 1 (SCA1), which is characterized by cerebellar degeneration and progressive ataxia (Orr *et al.* 1993). Because of this, ataxin-1 has been studied extensively in the context of disease, while less is known about its endogenous function.

To look at the endogenous function of a protein, it helps to see what happens when that protein is absent. While rare, there are several reported cases of humans born with chromosomal deletions that include *ATXN1*, who all presented with developmental delays and autism spectrum disorders (Celestino-Soper *et al*. 2012). In addition, when *ATXN1* is knocked out in mice, the animals are largely healthy aside from performing poorly on the Morris Water Maze (Matilla *et al.* 1998). The Morris Water Maze is a test of spatial learning and memory in which mice are placed in opaque water and must learn the location of a submerged hidden platform to escape the water. While wild-type mice learned to find the platform faster with training, the ataxin-1 knockout mice never acquired the task even after 17 days of training. Unsurprisingly, in a probe trial where the platform was taken away, the knockout mice searched all parts of the maze equally with no apparent memory of where the platform used to be, while their WT littermates preferentially searched in and around the former location of the platform. These human deletion case reports and mouse studies both suggest that ataxin-1 may be important for normal cognition.

Ataxin-1 also appears to play a role in cognition in several disease states. Patients with SCA1, which arises from polyglutamine expansion in ataxin-1, can develop cognitive symptoms including memory loss and executive function deficits in addition to the more well-studied motor impairments caused by the disease (Bürk *et al*. 2003; Kish *et al*. 1988). This is especially noticeable in aggressive juvenile-onset cases, where cognitive symptoms are more pronounced and can precede other symptoms (Zoghbi *et al*. 1988). Notably, polymorphism in ataxin-1 has also been linked to Alzheimer's risk (Bertram *et*

al. 2008). In mice, knocking in an expanded *ATXN1* allele with 154 repeats leads to many features of SCA1, including progressive ataxia and early death (Watase *et al*. 2002). Like ataxin-1 knockout mice, these *Atxn1*$^{154Q/2Q}$ mice also display cognitive deficits, as measured by the Morris water maze (Watase *et al*. 2002) and fear conditioning (Watase *et al*. 2007, 2002).

The fact that both deletion and polyglutamine expansion of *ATXN1* cause cognitive deficits raises a question regarding *ATXN1*'s role in cognition: Are the cognitive deficits in SCA1 due to toxic gain-of-function in the polyglutamine-expanded ataxin-1 or to loss of endogenous ataxin-1 function? This is an important and pressing question to ask—the ataxia and premature death associated with polyglutamine expansion in ataxin-1 are thought to be toxic gain-of-function effects, because *Atxn1*$^{-/-}$ mice only develop mild motor incoordination and do not die prematurely (Matilla *et al.* 1998). In addition, turning off the expression of the mutant allele in conditional SCA1 trasngenic mice alleviates disease phenotypes (Zu *et al.* 2004). Because of this, promising preclinical treatments for SCA1 aim to reduce the levels of mutant ataxin-1 protein to correct these features of the disease (Harry Orr, personal communication). However, these treatments cannot yet distinguish between wild-type and mutant ataxin-1 and are likely to cause some degree of ataxin-1 loss of function. The fact that *Atxn1*$^{-/-}$ mice have cognitive deficits suggests that the loss of ataxin-1 function caused by these treatments could have detrimental cognitive side effects.

A second open question is which brain region(s) contribute to *ATXN1's* role in nonmotor functions, given that it is expressed throughout the brain (Banfi *et al.* 1996) but mainly

studied in the context of the cerebellum. The cerebellum could, in fact, be responsible for these effects of *ATXN1*—although it was traditionally thought to specialize in motor coordination, more recent evidence suggests that the cerebellum also plays a critical role in cognition and mood (for review see Koziol *et al.* 2014 and Bodranghien *et al.* 2016). One line of evidence comes from anatomical and functional connectivity. For example, the posterior cerebellum is heavily connected to association and limbic areas (Buckner *et al*. 2011; Stoodley and Schmahmann 2010, 2009), is activated during a wide range of cognitive tasks without a heavy motor component (Keren-Happuch *et al*. 2014), and atrophies alongside the frontal cortex in Alzheimer's disease and frontotemporal dementia (Guo *et al*. 2016). This is supported by case studies in patients with lesions affecting the posterior cerebellum. These patients may develop the "cerebellar cognitive affective syndrome," characterized by impaired executive function, poor visuospatial cognition, linguistic abnormalities, and personality changes such as impulsive behavior (Schmahmann and Sherman 1998; Stoodley *et al*. 2016; Tedesco *et al*. 2011). These observations have led to the hypothesis that the cerebellum modulates activity from diverse cortical areas. In this model, damage to the cerebellum would not only cause dysregulated movement but also dysregulated mood and cognition. The cognitive and mood features of SCA1 could therefore be a direct result of cerebellar degeneration.

It is difficult to determine the cerebellar contribution to cognition in human SCA1 because degeneration is not confined to the cerebellum in SCA1, and other conditions with more restricted cerebellar pathology may not be representative of the mechanisms of SCA1. For example, a recent longitudinal investigation compared human patients with

SCA1, which affects a variety of extracerebellar regions, with patients with SCA6, in which degeneration is largely confined to the cerebellum (Moriarty *et al.* 2016). They found that both SCA1 and SCA6 patients developed cognitive deficits as their cerebellar motor symptoms progressed, but that cognitive deficits progressed faster in SCA1 patients. This suggests that the cerebellum plays a major role in cognitive deficits in SCA1, but may not be the only contributor. However, the differences observed in cognitive symptoms between the SCA1 and SCA6 patients could be due to a number of factors, such as faster clinical progression in SCA1 patients, small sample size, different cognitive tests used, or differences in the ages of patients tested. Mouse studies would allow us to better control for many of these factors, but to our knowledge no cognitive study has ever been published on mice in which *ATXN1* is manipulated only in the cerebellum.

Even less studied than *ATXN1*'s role in cognition is its role in mood. Patients with SCA1 experience high rates of depression compared to the general population (Klinke *et al.* 2010; Lo *et al.* 2016; Schmitz-Hübsch *et al.* 2011), which can seriously impact their quality of life. It is unknown whether this is a direct effect of polyglutamine-expanded ataxin-1 or the result of living with a terminal disease; knowing this could help with planning approaches to treat depression in SCA1 patients. Mouse studies can help clarify this. Indeed, a recent study showed that conditional forebrain knockouts of either *Atxn1L* (a paralogue of *ATXN1*) on an *Atxn1*$^{+/-}$ or *Atxn1*$^{-/-}$ background or of *Capicua* (a transcriptional regulator that interacts with *ATNX1)* leads to decreased anxiety-like phenotypes on the elevated plus maze and open field tests (Lu *et al*. 2017). However,

mouse studies focusing on the effect of *ATXN1* itself on mood, particularly depression, are lacking.

Thus, while both mouse and human studies hint at a role for ataxin-1 in cognition and mood, several major open questions remain: the mechanisms of *ATXN1's* effect—including the role of loss versus gain of function and the role of the cerebellum versus other areas—and whether *ATXN1* affects mood in mice at all. This thesis research is aimed at better characterizing the learning, memory and mood deficits in *ATXN1* mutant mice in order to provide answers to these questions.

In order to investigate these questions, we have used several different strains of mice.

First, global *ATXN1* knockout (*Atxn1*$^{-/-}$) mice, in which the majority of the coding region (exon 8) of *ATXN1* has been removed, can provide us with insight into which phenotypes result from a loss of endogenous *ATXN1* function. Previous studies have shown that homozygous *Atxn1* knockout mice are deficient on the Morris water maze (Matilla *et al.* 1998), but to our knowledge there is no published data on cognition in heterozygous *Atxn1* knockout mice despite the recent interest in reducing ataxin-1 levels for SCA1 treatment. Heterozygous *Atxn1*$^{+/-}$ mice are a convenient model for testing whether a 50% reduction in ataxin-1 protein will impact cognition as a potential side effect, and we have therefore included them in all behavioral tests alongside their WT and *Atxn1*$^{-/-}$ littermates.

Second, *Atxn1*$^{154Q/2Q}$ knockin mice, a mouse model of SCA1 in which one allele of the *ATXN1* gene contains a long CAG tract with 154 repeats, can provide insight into which phenotypes result from a toxic gain of function in polyglutamine-expanded ataxin-1

(Watase *et al.* 2002). *Atxn1*$^{154Q/2Q}$ mice show progressive ataxia, early death, and cognitive deficits on the Morris water maze and fear conditioning (Watase *et al.* 2002). We also tested *Atxn1*$^{78Q/2Q}$ knockin mice, in which the CAG tract is 78 repeats long (Lorenzetti *et al.* 2000). These mice do not develop severe progressive ataxia or early death, but their repeat is more similar to that seen in humans, in which the largest recorded CAG tract in *ATXN1* was 82 repeats long (Zoghbi *et al*. 1988). If *Atxn1*$^{78Q/2Q}$ mice do develop cognitive deficits, it would be an interesting illustration of repeat length affecting movement, cognition, and mood differently. These mice would also provide a more biologically and clinically relevant model for molecular biology studies moving forward. Both *Atxn1*$^{154Q/2Q}$ mice and *Atxn1*$^{78Q/2Q}$ mice can reveal phenotypes which may be more relevant to SCA1 than other diseases. In addition, by comparing them to *Atxn1*$^{-/-}$ mice tested by the same experimenter using the same procedures in the same laboratory, we can more easily predict which of these phenotypes arise primarily through gain versus loss of function.

Finally, we also investigated cognition and mood in *Pcp2-Atxn1[82Q]* mice, another SCA1 model in which an expanded *ATXN1* allele with 82 CAG repeats is overexpressed selectively in cerebellar Purkinje neurons under the *Purkinje cell protein 2 (Pcp2)* promoter. These mice develop progressive ataxia but not premature death, suggesting that ataxia arises predominantly through cerebellar dysfunction but that this cerebellar dysfunction alone is not fatal (Burright *et al.* 1995). Similarly, they can help reveal which brain regions are important to *ATXN1*'s role in cognition and mood by providing us with a strain in which mutant *ATXN1* is confined to cerebellar Purkinje neurons.

We tested learning and memory in these four strains of mice using the Barnes maze and context fear conditioning. We also present the first studies on mood in many of these strains, using the elevated plus maze to test for an anxiety-like phenotype and the forced swim and sucrose preference tests to test for a depression-like phenotype. In addition, we show that *ATXN1* plays a role in regulating adult hippocampal neurogenesis, a crucial hippocampal function which may underlie some of the learning and memory phenotypes we observe.

Results

Important note: We experienced some technical difficulties in gathering this data, most notably leaks and spills interfering with collection of sucrose preference data and poor performance of wild-type mice on tests of learning and memory in some cohorts. Most individual mice which experienced obvious problems which interfered with the test have been excluded from analysis (see the methods sections of each chapter for details), but not all of the data have been thoroughly vetted for inclusion at the time of writing. These results should therefore be considered preliminary and subject to change.

Chapter 1: Cognition and mood in *Atxn1*$^{-/-}$ mice

Introduction

Evidence from both human and mouse studies points to a role for *ATXN1* in cognition. In humans, chromosomal deletions containing *ATXN1* cause developmental delays and autism spectrum disorders (Celestino-Soper *et al.* 2012). Patients with spinocerebellar ataxia type 1 (SCA1), which is caused by expansion of a CAG repeat in *ATXN1*, are also prone to developing mild cognitive deficits (Bürk *et al.* 2003). In mice, deletion of the majority of the coding region of the *ATXN1* gene (exon 8) leads to severe deficits on the Morris water maze, a test of spatial learning and memory (Matilla *et al.* 1998). Here we attempt to replicate these results on the Barnes maze and context fear conditioning, including both homozygous *(Atxn1*$^{-/-}$*)* and heterozygous *(Atxn1*$^{+/-}$*) ATXN1* knockout mice to test whether a partial reduction in *ATXN1* can also cause cognitive deficits.

In addition to cognition, *ATXN1* may also play a role in mood. We therefore performed standard tests of depression- and anxiety-like phenotypes on *Atxn1*$^{-/-}$ and *Atxn1*$^{+/-}$ mice to better determine what role *ATXN1* plays in regulating multiple types of mood.

Methods

Mice. Atxn1$^{-/-}$ mice (Matilla *et al.* 1998) on a C57/Bl6 background were a gift from the laboratory of Dr. Huda Zoghbi. Mice were housed in a temperature- and humidity-controlled room on a 12 hour light/12 hour dark cycle with access to food and water *ad libitum*. Behavioral experiments were performed during the light phase of the cycle. 15 WT, 22 *Atxn1*$^{+/-}$, and 11 *Atxn1*$^{-/-}$ mice of both sexes, age 8-12 weeks, were used. Mice

went through a battery of tests, with the same order of testing for all mice (in order: elevated plus maze, forced swim test, Barnes maze, fear conditioning, sucrose preference). All animal experiments were performed in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and the University of Minnesota Institutional Animal Care and Use Committee.

Barnes maze. The maze was a white circular platform 91 cm in diameter with 20 5-cm circular holes spaced evenly around the edge, raised approximately 92 cm above the floor. One of the holes led to a 5 cm wide x 11 cm long x 5 cm deep opaque box (the "escape box") and the other 19 were covered. The testing room had visual cues on the walls to serve as landmarks, and all objects in the room were kept in the same places for every trial. During the training trials, the mouse was placed under an opaque plastic bucket in the center of the maze with the lights turned off for approximately 10 seconds. The lights were turned on, the bucket was removed, and the mouse was allowed to explore the maze for 3 minutes or until it entered the escape box, after which the escape box was covered and the mouse allowed to sit in it for approximately 1 minute before being returned to its home cage. The position of each mouse was tracked using AnyMaze software. Mice which did not enter the escape box within 3 minutes were gently guided to it. Mice were exposed to the maze for four consecutive training days (four trials per day with an intertrial interval of approximately 15 minutes). Training day data is reported as path length (distance traveled before entering the escape hole) and analyzed by two-way repeated measures ANOVA. A probe test was conducted 24 hours after the last training session. For the probe test, the escape hole was covered and each mouse was

allowed to explore the maze freely for 90 seconds. The time spent in each quadrant of the maze was recorded, and the amount of time spent in the goal quadrant (the quadrant centered on the goal hole) was analyzed by one-way ANOVA.

Search strategies on the training days were classified automatically into six categories using the Barnes maze unbiased strategy (BUNS) classification tool (Illouz *et al.* 2016). Each strategy was also assigned a "cognitive score" between 0 and 1 (Direct=1.0, Corrected=0.75, Long correction=0.5, Focused search=0.5, Serial=0.25, Random=0.0). This scoring scale was taken from Illouz *et al.* 2016 and is based on how similar the strategy is to the optimal "Direct" strategy, as determined by a k-means clustering analysis. In order to compare learning rates between groups, cognitive scores for each mouse on each of the 16 training trials were plotted in GraphPad Prism 7.0. Linear regression was performed for each group and the slopes and elevations of the lines were compared using Prism's Analysis function.

Context fear conditioning. Conditioning took place in chambers with a floor consisting of stainless steel rods through which shocks were delivered (Med Associates #ENV-008-FPU-M). On day 1, mice were placed in the chambers for a 10-minute period during which they received five foot shocks (0.70 mA, 2-second duration). Freezing during the 60 seconds after each shock was quantified automatically using VideoFreeze software (freezing was defined as a motion index ≤15 lasting ≥500 ms). 24 hours after the initial conditioning, mice were returned to the same chambers with the shock generators turned off and freezing behavior was monitored for 3 minutes. 1-2 hours after being placed in the conditioned context, mice were placed in a second context for 3 minutes to measure

baseline freezing. The baseline context used the same chambers but differed from the conditioned context in floor texture (smooth plastic versus metal rods), shape (curved plastic wall versus square metal wall), and odor (0.5% vanilla extract versus 33% Simple Green). The white house lights and NIR lights were on during both conditioning and recall. Acquisition of freezing responses is reported as percent freezing in the 60-second period following each of the 5 foot shocks, analyzed by two-way repeated measures ANOVA. 24-hour recall is reported as percent freezing in each context over the 3-min test period, analyzed by two-way repeated measures ANOVA.

Elevated plus maze. The maze was approximately 76 cm across and consisted of two open arms (6.3 cm x 34.2 cm) and two closed arms (6.3 cm x 34.2 cm with 19 cm tall opaque walls) extending from a 6.3 cm x 6.3 cm center area, raised 96 cm above the floor. The surrounding room was dark and the maze was lit by overhead lights. Light intensity in the open arms was 45-50 lux, and in the closed arms was 3-6 lux. Each mouse was placed in the center of the maze facing an open arm and allowed to explore for 5 minutes. During this time, the mice were tracked automatically using AnyMaze software and the time spent in each zone (open arms, closed arms, center area) was recorded. The maze was cleaned with 70% ethanol before each mouse. The time spent in each zone was analyzed using one-way ANOVA.

Forced swim test. Mice were placed in a transparent cylindrical container (diameter 18.4 cm, height 23.5 cm) filled to a depth of approximately 15 cm with water at 24-26°C. They swam for six minutes before being removed from the water and placed in a cage containing absorbent material on a heating pad to dry off. Time immobile was tracked

automatically using AnyMaze software (immobility was defined as a period of 85% or greater immobility lasting 250 ms or longer). The time spent immobile in seconds during the last 4 minutes of the test is presented here, analyzed by one-way ANOVA.

Sucrose preference test. Mice were singly housed in cages with cotton nestlets (instead of shredded paper, which is standard in our mouse facility but can easily sit against water bottle spouts and absorb water) in order to allow for accurate measurement of each mouse's water and sucrose consumption. Mice were acclimated to solitary housing for 3 days before the test. On the first day of testing, each mouse was given two bottles of water to test for any bias towards one side of the cage. On each day following this, each mouse was given one bottle containing water and another one containing either 2% or 4% sucrose. Bottles were weighed every 24 hours to measure the amount of liquid consumed. Results for the 4% sucrose day, for which we saw the greatest differences between groups, are reported here, analyzed by two-way ANOVA.

Excluded mice. No mice were excluded from the Barnes maze, although individual training trials were removed whenever the software failed track the mouse's location. One *Atxn1*$^{+/-}$ and one *Atxn1*$^{-/-}$ mouse were excluded from fear conditioning analysis because of excessive handling (they were removed from the chambers and returned after calibrating the camera, a step which should have been done before putting the mice in the chambers). On the elevated plus maze, one WT mouse was excluded after being moved to a new cage with new cagemates shortly before testing; one *Atxn1*$^{-/-}$ mouse failed to explore the maze; one WT mouse jumped off of the maze; and one *Atxn1*$^{+/-}$ mouse was excluded because the room lights came on during testing. One *Atxn1*$^{+/-}$ mouse was

excluded from the forced swim test analysis because the software did not track it well. On the sucrose preference test, one *Atxn1*$^{-/-}$ and one *Atxn1*$^{+/-}$ mouse were excluded due to large spills discovered in the cage. In addition, one large cohort showed relatively high total volumes consumed and is suspected of experiencing spills, but was left in for the current analysis as no wet spots were observed in the cages.

Results

We tested whether loss of ataxin-1 affects cognition in both homozygous (*Atxn1*$^{-/-}$) and heterozygous (*Atxn1*$^{+/-}$) *ATXN1* knockout mice. Mice were tested in a battery of tests from 8-12 weeks of age. This age is both convenient for testing and relevant to potential side effects in preclinical testing, as several SCA1 model mouse lines begin to develop ataxia by approximately 12 weeks of age.

In order to test cognition in *Atxn1*$^{-/-}$ mice we used two different tests: the Barnes maze and context fear conditioning. The Barnes maze is a dry-land version of the Morris water maze in which mice must learn and remember the location of an opening to escape an open field (Barnes 1979). We chose the Barnes maze over the Morris water maze for two reasons. First, it only requires walking and not swimming, making it less demanding on mice which may be experiencing subtle motor deficits. Second, the Barnes maze is both less stressful on mice and less sensitive to test-related stress (Harrison, Hosseini, and McDonald 2009).

For the Barnes maze, mice were subjected to four training trials a day for four days to learn the location of the escape box, followed by a probe trial on the fifth day where the escape box was removed to test their recall. Figure 1A shows the performance of *Atxn1*$^{-/-}$

mice on each of the training days, presented as path length (the distance traveled by the mouse before reaching the escape box). A shorter path length indicates a more efficient route to the escape box and better memory. We chose to use this measure rather than the more commonly reported latency to escape (the time it takes mice to reach the escape box) because there is some evidence that *Atxn1*$^{-/-}$ mice may have subtle motor deficits, which could impact their running speed and thus their latency (Matilla *et al.* 1998). Indeed, we observed a significant effect of genotype on speed during the training days (two-way RM ANOVA, $F_{(2,45)} = 4.741$, P=0.0135, n=15 WT, 22 *Atxn1*$^{+/-}$, and 11 *Atxn1*$^{-/-}$ mice)—a potentially serious confound for latency.

Mice in each group improved in path length over the four training days, as seen by a significant main effect of day in a 2-way repeated measures ANOVA ($F_{(3,135)} = 28.38$, $p<0.0001$) with each group showing significant differences between day 1 and day 4 using Tukey's post hoc test (WT day 1 average 2.333 m, day 4 0.9299 m, $p<0.0001$, n=15; *Atxn1*$^{+/-}$ day 1 average 2.645 m, day 4 0.9552 m, $p<0.0001$, n=22; *Atxn1*$^{-/-}$ day 1 average 2.569 m, day 4 1.358 m, p=0.0019, n=11). This indicates that *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice are motivated to find the escape hole and capable of acquiring the task. Although the *Atxn1*$^{-/-}$ mice had the highest mean path length on day 4, this trend was not significant, as we detected no significant effect of genotype ($F_{(2,45)}=2.385$, p=0.1036) or of a genotype x day interaction ($F_{(6,135)}=0.7585$, p=0.6038) on path length.

For the probe trial, the escape hole was covered and the mice were allowed to explore the maze for 90 seconds. Memory is represented by the amount of time the mouse spends in each quadrant of the maze; a mouse which remembers the former location of the escape

box will spend more time looking for it in the correct goal quadrant than in the other three quadrants. We detected a significant effect of genotype on time spent in the goal zone by one-way ANOVA ($F_{(2, 45)}$ = 11.35, p=0.0001). *Atxn1*$^{-/-}$ mice—but not *Atxn1*$^{+/-}$ mice—performed significantly worse than WT (average time in goal quadrant: WT 37.62 s, *Atxn1*$^{+/-}$ 41.26 s, *Atxn1*$^{-/-}$ 25.58 s, p = 0.4521 for WT vs *Atxn1*$^{+/-}$ and 0.0042 for WT vs *Atxn1*$^{-/-}$, Tukey's multiple comparisons test, Figure 1B).

The fact that *Atxn1*$^{-/-}$ mice improve over the training days but still perform poorly on the probe trial can be interpreted in multiple ways. They may have learned the location of the escape box quickly over the four trials each day but failed to remember it 24 hours later on the probe trial, or they may have learned to find the escape box through some means other than using spatial cues in the room. Indeed, wild-type mice can improve on multiple measures of Barnes maze performance over training even when the escape box is moved to a new semi-random location each trial (O'Leary and Brown 2013). In this case, they often develop a serial search strategy, in which they simply run around the edge of the maze checking each hole in series until the correct one is found. This allows them to efficiently search the maze even without learning the location of the goal relative to spatial cues. To test for this possibility, we used the Barnes maze unbiased strategy (BUNS) classification tool developed by Illouz *et al.* (2016) to automatically classify the search strategy used by each mouse on each training trial. Strategies were sorted into six categories: 1) Direct, in which the mouse takes the most efficient path directly to the goal; 2) Corrected, in which the mouse first goes toward one of the holes adjacent to the goal and makes a correction to reach the goal; 3) Long correction, in which the mouse

first moves toward a hole far from the goal and then turns around and goes directly to the goal; 4) Focused search, in which the mouse scans a larger area near the goal, 5) Serial, in which the mouse goes around the maze checking each hole in series, and 6) Random, in which the mouse takes a long and inefficient path with no obvious pattern.

Figure 1C shows example path plots from our mice using each of these strategies, as well as group data over the four training days (16 trials total) for wild type, *Atxn1*$^{+/-}$, and *Atxn1*$^{-/-}$ mice. Nearly all mice took a random path on the first trial, when they were naive to the maze. This was quickly replaced by more efficient strategies as mice learned the task. Mice tended to pass through a serial search stage, which was later replaced partially by strategies which rely on knowledge of where the hole is located in space (*i.e.* Direct, Corrected, Long correction, and Focused search).

In order to quantify the rate at which the mice learned the spatial strategies, each strategy was assigned a "cognitive score" based on how similar it is to the optimal "Direct" strategy (Direct=1.0, Corrected=0.75, Long correction=0.5, Focused search=0.5, Serial=0.25, Random=0.0, see Illouz *et al.* 2016 for details). The cognitive score for each mouse was plotted as a function of trial number and regression lines were fitted to each group (WT: $Y = 0.02549*X + 0.1124$, *Atxn1*$^{+/-}$: $Y = 0.02494*X + 0.05666$, *Atxn1*$^{-/-}$: $Y = 0.02024*X + 0.08211$). Although the *Atxn1*$^{-/-}$ group had the lowest slope, this difference was not statistically significant ($F_{2,751}=0.4775$, $p=0.6205$). This indicates that all three groups of mice learned more effective search strategies at similar rates. There was, however, a statistically significant difference in the elevations of the lines ($F_{2,753}=5.01$, $p=0.0069$). This could indicate that, while all groups improved at the same

rate, the WT mice were using better strategies overall across all timepoints, and may warrant further investigation.

These results suggest that *Atxn1*$^{-/-}$ mice can learn to use spatial cues to find the goal, but do not retain this information well on the probe trial, in contrast with previous research using the Morris water maze which suggested that they never acquire the task during training (Matilla *et al.* 1998). The discrepancy between our findings and those of Matilla *et al.* may be due to differences in the two tests used: for example, in the Barnes maze, all holes are located around the edge of the maze, allowing all possible goal locations to be searched efficiently, while in the Morris water maze the goal could be in a wider variety of locations.

We used the Barnes maze because it is similar in design to the Morris water maze, which revealed severe learning and memory deficits in both *Atxn1*$^{-/-}$ mice and *Atxn1*$^{154Q/2Q}$ mice in previous studies (Matilla *et al.* 1998; Watase *et al.* 2002). However, it is not necessarily the best test of learning and memory in these mice, due to its dependence on motor ability and the possibility of solving the task through nonspatial strategies. Because of this, we also tested learning and memory in *Atxn1*$^{-/-}$ mice with context fear conditioning. In context fear conditioning, mice receive footshocks in one context and are tested for freezing responses 24 hours later in both the original conditioned context (a square space with metal walls and a metal grate on the floor, scented with Simple Green) and a control context (with a curved plastic wall and flat plastic floor, scented with vanilla extract). Freezing in the conditioned context but not the control context in a recall test 24 hours after conditioning indicates learning.

Figure 1D shows the acquisition of freezing responses during conditioning in WT, *Atxn1*$^{+/-}$, and *Atxn1*$^{-/-}$ mice. All groups greatly increased their freezing over the course of conditioning (significant effect of shock number by 2-way repeated measures ANOVA, $F_{(4,172)}$=95.85, p <0.0001, n=15 WT, 21 *Atxn1*$^{+/-}$, and 10 *Atxn1*$^{-/-}$ mice), indicating that they are able to feel the shock and freeze in response to multiple inescapable shocks. However, there was also a significant effect of genotype on freezing ($F_{(2,43)}$=3.576, p=0.0366). *Atxn1*$^{-/-}$ mice froze less during the last part of conditioning than *Atxn1*$^{+/-}$ mice (45.19% freezing in *Atxn1*$^{+/-}$ during the 60 s following the last shock vs 29.99% in *Atxn1*$^{-/-}$ mice, p=0.0311, Tukey's multiple comparisons test), indicating a possible change in freezing behavior which complicates the interpretation of 24-hour recall results.

When tested for recall 24 hours later (Figure 1E), all groups froze significantly more in the conditioned context than in a control context (significant main effect of context by two-way repeated measures ANOVA, $F_{(1,43)}$=85.96, p<0.0001, n=15 WT, 21 *Atxn1*$^{+/-}$, and 10 *Atxn1*$^{-/-}$ mice). There was also a significant effect of genotype ($F_{(2,43)}$=6.061, p=0.0048) with the *Atxn1*$^{-/-}$ mice freezing less than wild-type in the conditioned context (WT average 40.92% freezing, *Atxn1*$^{-/-}$ average 19.7% freezing, p=0.0088, Sidak's multiple comparisons test). There was a trend towards *Atxn1*$^{-/-}$ mice freezing less in the baseline context as well, but this did not reach statistical significance (WT average 15.98% freezing, *Atxn1*$^{-/-}$ average 4.9% freezing, p=0.3033, Sidak's multiple comparisons test). Heterozygous *Atxn1* knockout mice did not differ from wild type in either the conditioned context (WT average 40.92% freezing, *Atxn1*$^{+/-}$ average 46.14% freezing, p=0.7488, Sidak's multiple comparisons test) or the baseline context (WT average

15.98% freezing, *Atxn1*$^{+/-}$ average 18.12% freezing, p=0.9758, Sidak's multiple comparisons test). These results may indicate a memory deficit in the *Atxn1*$^{-/-}$ mice but not the *Atxn1*$^{+/-}$ mice, in agreement with the Barnes maze results. However, caution should be used when interpreting the fear conditioning results alone, given the tendency of *Atxn1*$^{-/-}$ mice to freeze less during conditioning and the trend towards *Atxn1*$^{-/-}$ mice freezing less in the baseline context.

In addition to cognitive tests, we also examined mood in *ATXN1*-deficient mice using three different tests: the elevated plus maze to test for an anxiety-like phenotype (Handley and Mithani 1984), and the forced swim (Porsolt, Bertin, and Jalfre 1977) and sucrose preference tests (Willner *et al.* 1987) to test for a depression-like phenotype.

The elevated plus maze consists of a plus-shaped platform with two "closed" arms (which are enclosed by tall opaque walls and are dimly lit) and two "open" arms (which have no walls and are more brightly lit). As small prey animals, mice prefer dark enclosed spaces to brightly lit open spaces and are therefore expected to be more comfortable in the closed arms. However, they will still explore the novel environment of the open arms to some extent. A reduction in time spent exploring the open arms is thought to indicate an anxiety-like phenotype. On the elevated plus maze, we saw a significant effect of genotype on open arm time (One-way ANOVA, $F_{(2,39)} = 5.341$, p=0.0070, n=13 WT, 21 *Atxn1*$^{+/-}$, and 9 *Atxn1*$^{-/-}$ mice, Figure 2A). A*txn1*$^{-/-}$ mice spent *more* time in the open arms than heterozygous knockout mice (*Atxn1*$^{-/-}$ average 40.72 sec, *Atxn*$^{+/-}$ average 15.88 sec, p=0.068, Holm-Sidak's multiple comparison test), indicating a possible reduction in anxiety-like phenotype, though this trend was not statistically

significant relative to wild-type (*Atxn1*$^{-/-}$ average 40.72 sec, WT average 23.9 sec, p=0.1025, Holm-Sidak's multiple comparison test).

The forced swim test measures an animal's response to an inescapable negative situation, and is thought to relate to the predisposition to negative mood and pessimism seen in human depression. Mice are placed in water in a chamber they cannot climb out of and swim for several minutes. In this situation, the mice will spend periods of time struggling to escape and other periods of time floating immobile in the water. Increased immobility time indicates a shift from active coping strategies to passive coping strategies—one component of a depression-like phenotype. We saw a significant effect of genotype on immobility time by one-way ANOVA ($F_{(2,44)}$= 17.17, p<0.0001, n= 15 WT, 21 *Atxn1*$^{+/-}$, and 11 *Atxn1*$^{-/-}$ mice, Figure 2B). Interestingly, this was because *Atxn1*$^{-/-}$ mice were *less* immobile than their WT and *Atxn1*$^{+/-}$ littermates, indicating that *Atxn1* knockout in mice may alter mood in a less straightforward way than simply causing a depression-like phenotype (WT average 164.2 sec, *Atxn1*$^{+/-}$ average 160.6 sec, *Atxn1*$^{-/-}$ average 106.5 sec, p<0.0001 for *Atxn*$^{-/-}$ vs both WT and *Atxn1*$^{+/-}$, Holm-Sidak's multiple comparison test).

The sucrose preference test is thought to measure anhedonia, or lack of interest in pleasurable things, which is another feature of depression. Mice are given two bottles containing water and sucrose, respectively, and the amount of liquid they consume from each bottle is measured. Mice generally prefer sucrose over water; a reduction in sucrose preference indicates an anhedonia-like phenotype. Using two-way repeated measures ANOVA, we did not detect a significant effect of genotype ($F_{(2,19)}$ = 0.6058, P=0.5558) or of a genotype x sucrose interaction ($F_{(2,19)}$ = 0.5208, P=0.6023) on sucrose

consumption in *Atxn1*$^{+/-}$ or *Atxn1*$^{-/-}$ mice (Figure 2C). However, due to technical difficulties with leaking bottle lids for one large cohort of mice, we did not have enough data to draw strong conclusions.

Thus, based on our forced swim test and preliminary sucrose preference results, we may see an alteration in mood and/or coping strategy in *Atxn1*$^{-/-}$ mice, but this may not be related to a depression-like phenotype *per se.*

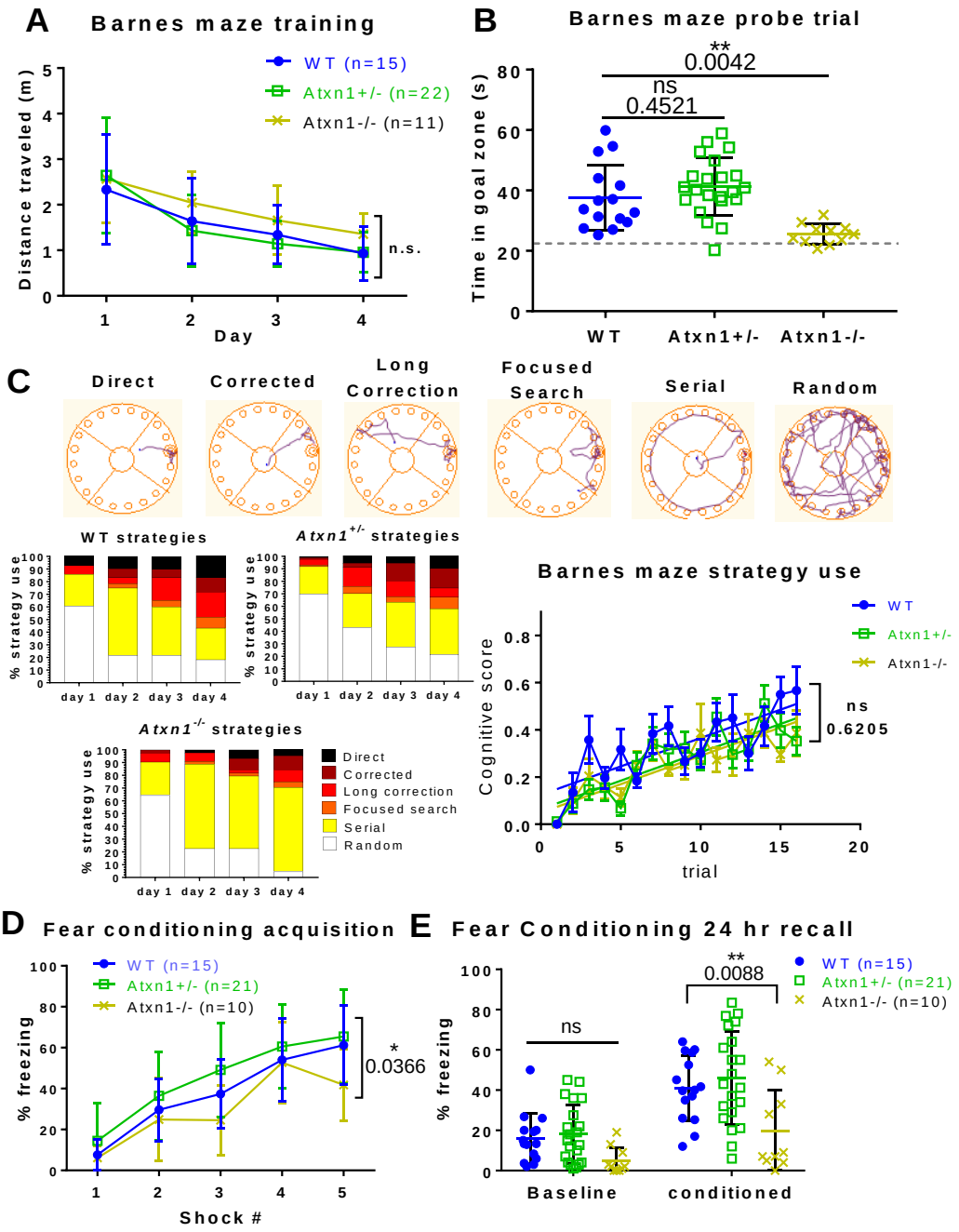

Figure 1. *Atxn1*$^{-/-}$ mice perform poorly on tests of learning and memory. A) Average path length for each of the four Barnes maze training days in 9-10-week-old WT, *Atxn1*$^{+/-}$, and *Atxn1*$^{-/-}$ mice. (2-way ANOVA). **B)** Time spent in the goal zone during the probe trial for 9-10-week-old WT, *Atxn1*$^{+/-}$, and *Atxn1*$^{-/-}$ mice (1-way ANOVA). **C)** Barnes maze search strategy use (left) and corresponding cognitive scores (right) over the four training days in 9-10 week old WT, *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice. **D)** Acquisition of freezing responses during context fear conditioning in 10-11 week old WT, *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice, reported as percent freezing during the 60-second interval after each shock (2-way ANOVA). **E)** Fear conditioning 24-hour recall in a baseline context and the conditioned context for 10-11 week old WT, *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice (2-way ANOVA). Data are presented as mean ± SD.

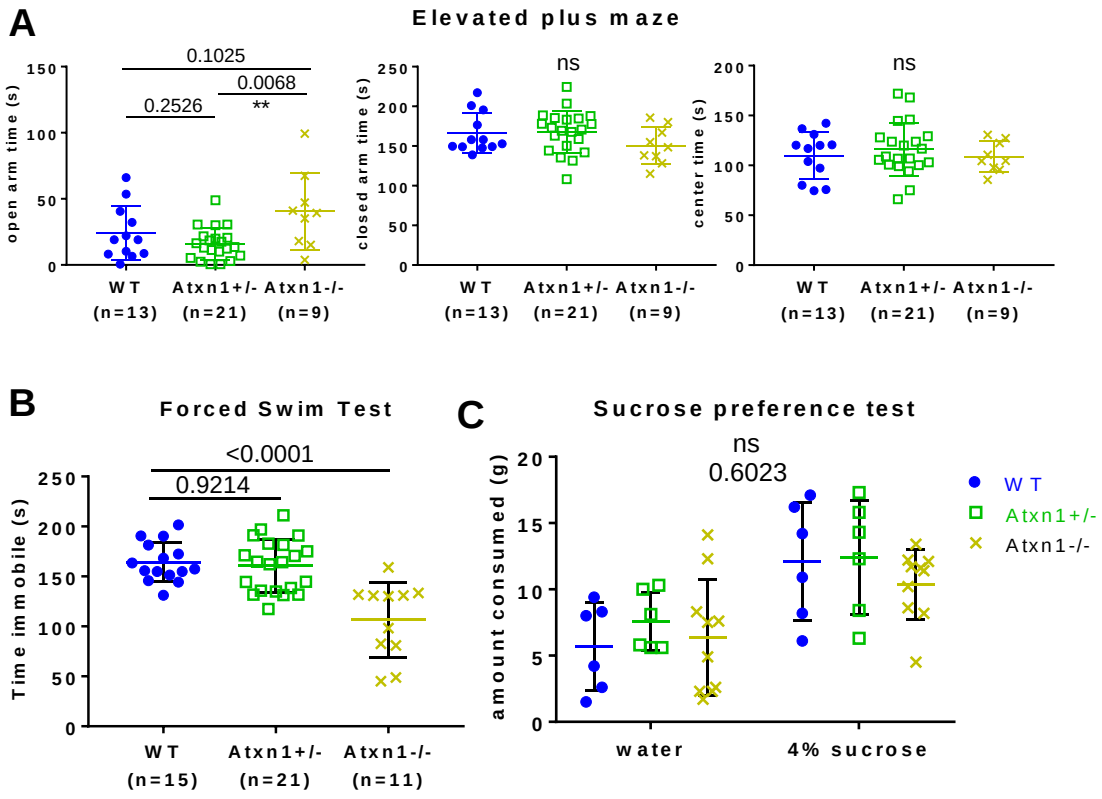

Figure 2. Mood in *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice. A) Elevated plus maze in 8-9 week old WT, *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice. The time spent in the open arms (left), closed arms (center) and center of the maze (right) is shown (one-way ANOVA). **B)** Forced swim test in 8-9 week old WT, *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice, reported as time spent immobile in the last 4 minutes of the test (one-way ANOVA). **C)** Water and sucrose consumption in the sucrose preference test in 11-12 week old WT, *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice (two-way ANOVA). Data are presented as mean ± SD.

Chapter 2: Cognition and mood in *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ knockin mice

Introduction

In order to test the effects of polyglutamine-expanded ataxin-1 on cognition and mood in mice, which may be relevant to SCA1 patients experiencing cognitive deficits and mood alterations, we used *Atxn1*$^{154Q/2Q}$ knockin mice. These mice have one copy of the mouse *ATXN1* gene with an extremely long CAG repeat (154 repeats) knocked into the endogenous locus, and one wild-type copy of mouse *ATXN1* with 2 CAG repeats (Watase *et al.* 2002). *Atxn1*$^{154Q/2Q}$ mice have previously been reported to have deficits on the Morris water maze (Watase *et al.* 2002, 2007) and fear conditioning (Watase *et al.* 2007). We aimed to confirm these previous findings of cognitive deficits in *Atxn1*$^{154Q/2Q}$ mice, compare them to *Atxn1*$^{-/-}$ mice from the same laboratory to determine the relative contributions of loss- versus gain-of-function mechanisms, and investigate the effects on mood. Because these mice develop progressive ataxia as they age, behavioral tests were conducted on mice between 8 and 12 weeks of age, in the hopes of testing them after they become old enough to complete the tests easily but before motor deficits can interfere with results.

One caveat of using *Atxn1*$^{154Q/2Q}$ mice is that their mutant ataxin-1 has a longer polyglutamine tract than any found in human SCA1 patients. They were engineered after *Atxn1*$^{78Q/2Q}$ knockin mice, modeled after the longest polyglutamine tract ever found in a human (82Q), failed to develop any noticeable motor features of SCA1 (Lorenzetti *et al.* 2000). However, cognition and mood have never been tested in *Atxn1*$^{78Q/2Q}$ mice to our

knowledge. We therefore included them in our study to determine whether the more natural and biologically relevant 78Q polyglutamine tract might be sufficient to cause cognitive deficits or mood disturbances. These mice were tested at the same ages as the *Atxn1*$^{154Q/2Q}$ mice to make it easier to compare them.

Methods

Animals. *Atxn1*$^{154Q/2Q}$ knockin mice (Watase *et al.* 2002) and *Atxn1*$^{78Q/2Q}$ knockin mice (Lorenzetti *et al.* 2000) were gifts from the laboratory of Dr. Harry Orr. They were generated as previously described on an FVB background and backcrossed onto a C57/Bl6 background. All experimental mice tested negative for the homozygous *Pde6* mutation which causes blindness in FVB mice. Male and female mutant mice from each strain as well as their wild-type littermates were used. Mice were 8-12 weeks old during testing.

Behavioral tests and statistical analysis. The Barnes maze, context fear conditioning, elevated plus maze, forced swim, and sucrose preference tests were conducted and analyzed as described above (see Chapter 1), except that t-tests were used in place of one-way ANOVA for comparisons between two groups. On the forced swim test, the data for the *Atxn1*$^{154Q/2Q}$ mice was not normally distributed and was analyzed with a Mann-Whitney test rather than a t-test.

Excluded mice: Atxn1$^{154Q/2Q}$. No mice were eliminated from the Barnes maze. Three mice (one WT and two *Atxn1*$^{154Q/2Q}$) were removed from the fear conditioning analysis because the chambers were not scented with Simple Green during conditioning. On the elevated plus maze, one *Atxn1*$^{154Q/2Q}$ mouse spent most of the test immobile in a closed

arm and was excluded from analysis. In addition, one WT and one *Atxn1*$^{154Q/2Q}$ mouse were excluded because of technical difficulties leading to excess handling (taking them off of the maze and putting them back on). On the forced swim test, one *Atxn1*$^{154Q/2Q}$ mouse was a poor swimmer and had to be rescued before the test was finished. One WT mouse was excluded because the software did not track it well. Two WT and one *Atxn1*$^{154Q/2Q}$ mouse were eliminated from the sucrose preference test because of leaking bottles.

Excluded mice: Atxn1$^{78Q/2Q}$. One WT mouse was excluded from the Barnes maze after freezing in place for most of the probe trial. During fear conditioning, two mice (one WT and one *Atxn1*$^{78Q/2Q}$) clung to the walls of the chambers and avoided shocks, two WT mice were excluded because the chamber was not scented with Simple Green during conditioning, and one WT mouse escaped from the testing rig after conditioning and was excluded from further testing. On the forced swim test, one WT and one *Atxn1*$^{78Q/2Q}$ mouse were excluded because the software did not track them, and four mice (two WT and two *Atxn1*$^{78Q/2Q}$) were excluded after they were taken out of the water and put back in when correcting technical difficulties. Three WT mice were excluded from the sucrose preference test due to leaks and spills.

Results

Atxn1$^{154Q/2Q}$ knockin mice

Figure 3 shows learning and memory in *Atxn1*$^{154Q/2Q}$ mice. Both wild-type and *Atxn1*$^{154Q/2Q}$ mice improved during training in the Barnes maze (Figure 3A), as evidenced by decreased path length over the four training days (significant effect of day by two-way

RM ANOVA, $F_{(3,81)}$ = 22.37 P<0.0001). Two-way ANOVA also revealed a significant effect of genotype ($F_{(1,27)}$ = 4.47 P=0.0439). *Atxn1*$^{154Q/2Q}$ mice showed a trend towards longer path length, but no single day was statistically significant after correcting for multiple comparisons. *Atxn1*$^{154Q/2Q}$ mice initially had lower speed than WT mice on day 1 (two-way ANOVA followed by Sidak's multiple comparisons test, WT average 0.06593 m/s, *Atxn1*$^{154Q/2Q}$ average 0.0399 m/s, p=0.0001), suggesting a potential effect of motor impairment. However, this is unlikely to have impacted their ability to learn the maze on subsequent days, as they reached roughly wild-type speeds by day 2 (two-way ANOVA followed by Sidak's multiple comparisons test, WT average 0.05919 m/s, *Atxn1*$^{154Q/2Q}$ average 0.05423 m/s, p=0.8778). In the probe trial (Figure 3B), *Atxn1*$^{154Q/2Q}$ mice performed poorly compared to WT (WT average 33.72 sec in goal zone, n=17, *Atxn1*$^{154Q/2Q}$ average 23.42 sec, n=13, p=0.0040, t-test), much like *Atxn1*$^{-/-}$ mice (see Figure 1B).

Analysis of search strategies on the four training days showed that, while wild-type mice adopted more spatial strategies (*i.e.* Direct, Corrected, Long correction, and Focused search) over time, *Atxn1*$^{154Q/2Q}$ mice relied heavily on a serial search strategy (circling the edge of the maze, checking each hole in succession until the escape box is found) even on the last trial on day 4 (Trial 16: WT 76% spatial strategies, 6% serial, and 18% random, n=17; *Atxn1*$^{154Q/2Q}$ 0% spatial strategies, 92% serial, and 8% random, n=13; Figure 3C). When strategy use was converted to cognitive scores and fitted with regression lines (WT: Y = 0.03224*X + 0.06324, *Atxn1*$^{154Q/2Q}$: Y = 0.01695*X + 0.04689), WT mice improved their scores at a significantly faster rate than *Atxn1*$^{154Q/2Q}$ mice (significantly

different slopes for the two regression lines, $F_{(1,474)}$=10.2, p=0.0015). This suggests that, while *Atxn1*$^{154Q/2Q}$ mice are capable of learning that the escape box exists and navigating to it as efficiently as possible with a non-spatial strategy, they never learn the location of the box relative to spatial cues in the room and therefore cannot find the goal hole when it is hidden in the probe trial. This is different from the *Atxn1*$^{-/-}$ mice, which show spatial learning but fail to retain the information.

During context fear conditioning (Figure 3D), *Atxn1*$^{154Q/2Q}$ mice acquired freezing responses similarly to wild-type mice, with no significant effect of genotype ($F_{(1,24)}$ = 1.46, p = 0.2387, n = 16 WT and 13 *Atxn1*$^{154Q/2Q}$) or genotype x shock interaction ($F_{(4,96)}$ = 2.118, P=0.0844) by two-way repeated measures ANOVA. However, when their recall was tested 24 hours later, they did not freeze any more in the conditioned context than in the baseline context—demonstrating a profound memory deficit relative to their wild-type littermates (baseline context: WT average 13.87% freezing, *Atxn1*$^{154Q/2Q}$ average 13.82% freezing, p>0.9999; conditioned context: WT average 33% freezing, *Atxn1*$^{154Q/2Q}$ average 13.45% freezing, p=0.0005, 2-way ANOVA followed by Sidak's multiple comparisons test, n= 15 WT and 11 *Atxn1*$^{154Q/2Q}$ mice, Figure 3E).

Figure 4 shows performance on the elevated plus maze, forced swim test and sucrose preference test in *Atxn1*$^{154Q/2Q}$ mice. On the elevated plus maze, *Atxn1*$^{154Q/2Q}$ mice spent less time in the center of the maze (WT average 100.7 sec, *Atxn1*$^{154Q/2Q}$ average 52.36 sec, p<0.0001, t-test, n=14 WT and 10 *Atxn1*$^{154Q/2Q}$ mice), coinciding with more time in the closed arms (WT average 155.1 sec, *Atxn1*$^{154Q/2Q}$ average 194 sec, p=0.0341, t-test, n=14 WT and 10 *Atxn1*$^{154Q/2Q}$ mice, Figure 4A). Time in the open arms varied widely

between individuals and was not statistically significantly different from wild-type (WT average 44.21 sec, *Atxn1*$^{154Q/2Q}$ average 53.65 sec, p=0.5485, t-test, n=14 WT and 10 *Atxn1*$^{154Q/2Q}$ mice). The increase in closed arm time, however, suggests an anxiety-like phenotype in the *Atxn1*$^{154Q/2Q}$ mice.

Atxn1$^{154Q/2Q}$ mice did not differ from their wild-type littermates in the forced swim test (WT median 161.2 sec, *Atxn1*$^{154Q/2Q}$ median 157.3 sec, p=0.2171, Mann-Whitney test, n=15 WT and 11 *Atxn1*$^{154Q/2Q}$ mice, Figure 4B). On the sucrose preference test (Figure 4C), we saw a significant genotype x sucrose interaction (two-way RM ANOVA, $F_{(1,25)}$ = 7.655, P=0.0105, n= 15 WT and 12 *Atxn1*$^{154Q/2Q}$ mice). *Atxn1*$^{154Q/2Q}$ mice consumed significantly *more* sucrose than wild-type littermates (WT average 9.319 g, *Atxn1*$^{154Q/2Q}$ average 12.79 g, p= 0.0028, Sidak's multiple comparisons test), while water consumption stayed the same (WT average 4.009 g, *Atxn1*$^{154Q/2Q}$ average 3.199 g, p= 0.6786, Sidak's multiple comparisons test).

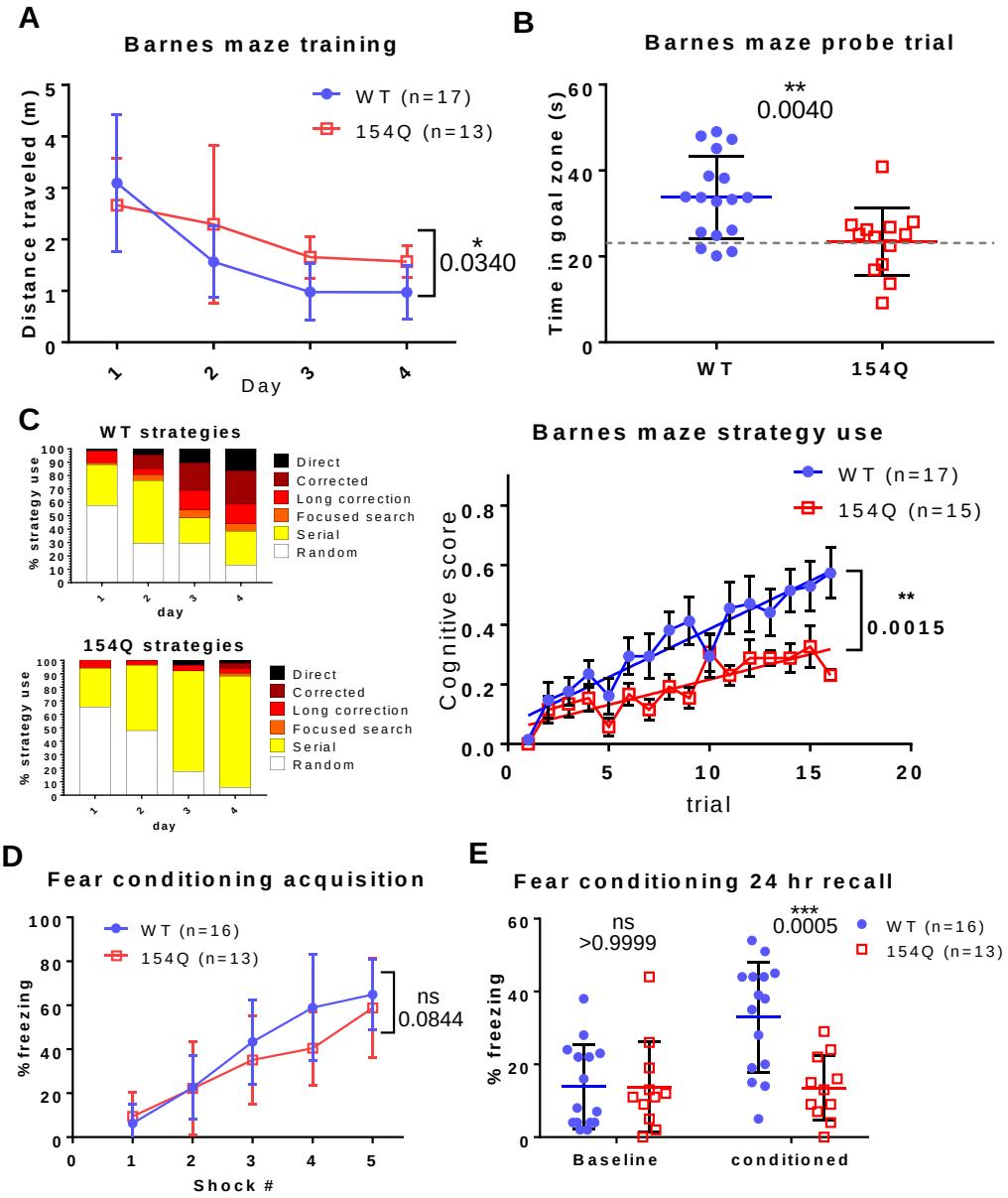

Figure 3. *Atxn1*$^{154Q/2Q}$ mice perform poorly on tests of learning and memory. A) Barnes maze average path length on each of the four training days for 9-10 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice (2-way ANOVA). **B)** Barnes maze probe trial (percent time spent in each zone) for 9-10 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice. (2-way ANOVA). **C)** Barnes maze search strategy use (left) and corresponding cognitive scores (right) over the four training days in 9-10 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice. **D)** Acquisition of freezing responses during context fear conditioning in 10-11 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice, reported as percent freezing during the 60-second interval after each shock (2-way ANOVA). **E)** Fear conditioning 24 hour recall in a baseline context and the conditioned context for 10-11 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice (2-way ANOVA). Data are presented as mean ± SD.

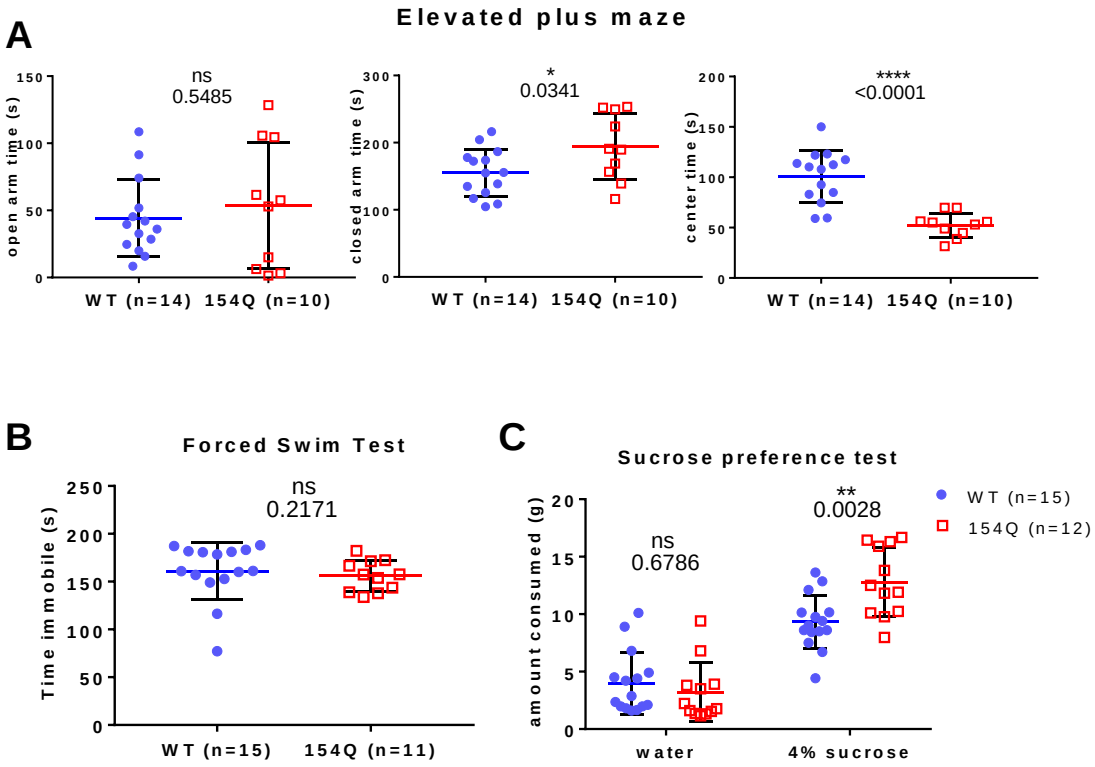

Figure 4. Mood in *Atxn1*$^{154Q/2Q}$ mice. A) Elevated plus maze in 8-9 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice. The time spent in the open arms (left), closed arms (center) and center of the maze (right) is shown. *Atxn1*$^{154Q/2Q}$ (t-test). **B)** Forced swim test in 8-9 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice, reported as time spent immobile in the last 4 minutes of the test. (Mann-Whitney test). **C)** Water and sucrose consumption in the sucrose preference test in 11-12 week old WT and *Atxn1*$^{154Q/2Q}$ knockin mice. (2-way ANOVA). Data are presented as mean ± SD.

Atxn1$^{78Q/2Q}$ mice

Figure 5 shows learning and memory results in *Atxn1*$^{78Q/2Q}$ mice. In contrast to the *Atxn1*$^{154Q/2Q}$ mice, the *Atxn1*$^{78Q/2Q}$ mice were not appreciably different from wild type during the Barnes maze (Figure 5A-5C), as measured by path length (no significant effect of genotype [$F_{(1,37)}$ = 0.0059, P=0.9393] or genotype x day interaction [$F_{(3,111)}$ = 1.288, P=0.2821] by two-way RM ANOVA, n= 23 WT and 15 *Atxn1*$^{78Q/2Q}$ mice) and strategy use during the training days (linear regression: WT: Y = 0.01851*X + 0.2491, *Atxn1*$^{78Q/2Q}$: Y = 0.0221*X + 0.1833, no significant difference between slopes of regression lines, $F_{(1,625)}$=0.4012, p=0.5267, or between elevations, $F_{(1,626)}$=1.845, p=0.1749), as well as recall on the probe trial (t-test, WT average 33.33 sec in goal zone, n=23; *Atxn1*$^{78Q/2Q}$ average 34.75 sec, n=15).

During context fear conditioning, *Atxn1*$^{78Q/2Q}$ mice acquired freezing responses similarly to wild type (no effect of genotype by 2-way repeated measures ANOVA; $F_{(1,40)}$ = 0.1055, P=0.7470, n = 20 WT and 20 *Atxn1*$^{78Q/2Q}$ mice, Figure 5D). When tested for recall 24 hours later, *Atxn1*$^{78Q/2Q}$ mice froze at wild-type levels in both the baseline and conditioned context (baseline context: WT average 18.28% freezing, *Atxn1*$^{78Q/2Q}$ average 19.69% freezing; conditioned context: WT average 56.87% freezing, *Atxn1*$^{78Q/2Q}$ average 58.88% freezing, no significant effect of genotype [$F_{(1,40)}$ = 0.0913, P=0.7641] or genotype x context interaction [$F_{(1,40)}$ = 0.0090, P=0.9249] by 2-way RM ANOVA, n=20 WT and 12 *Atxn1*$^{78Q/2Q}$ mice, Figure 5E). Together, these data suggest that *ATXN1* with a polyglutamine tract of 78 repeats is not sufficient to cause the same learning deficits as the version with 154 repeats.

Figure 6 shows tests of mood in *Atxn1*$^{78Q/2Q}$ mice. There was no significant difference between *Atxn1*$^{78Q/2Q}$ mice and wild-type on any measure of the elevated plus maze, suggesting that they do not have an anxiety-like phenotype. (WT average time in open arms = 49.01 ± 4.184 sec, *Atxn1*$^{78Q/2Q}$ average time in open arms = 53.41 ± 6.649 sec, p=0.1202; WT average time in closed arms = 122.3 ± 5.079 sec, *Atxn1*$^{78Q/2Q}$ average time in closed arms = 121.3 ± 4.718 sec, p=0.8964; WT average time in center=131.1 ± 5.169 sec, *Atxn1*$^{78Q/2Q}$ average time in center =125.2 ± 4.34 sec, p=0.3959; n=22 WT and 20 *Atxn1*$^{78Q/2Q}$ mice, t-tests, figure 6A.)

We also did not observe a depressive-like phenotype in *Atxn1*$^{78Q/2Q}$ mice as measured by the forced swim test (WT average time immobile=120.3 ± 7.271 sec, *Atxn1*$^{78Q/2Q}$ average time immobile = 116.6 ± 5.753 sec, p=0.1202, n=24 WT and 17 *Atxn1*$^{78Q/2Q}$ mice, t-test, Figure 6B). On the sucrose preference test (Figure 6C), we saw a significant effect of genotype by 2-way RM ANOVA ($F_{(1,39)}$ = 6.575, p=0.0143, n=20 WT and 21 *Atxn1*$^{78Q/2Q}$ mice). *Atxn1*$^{78Q/2Q}$ mice consumed *more* sucrose than wild-type (WT average 10.15 g, *Atxn1*$^{78Q/2Q}$ average 12.7 g, p=0.0161) with no statistically significant difference in water consumption (WT average 5.21 g, *Atxn1*$^{78Q/2Q}$ average 6.13 g, p=0.5498) much like the *Atxn1*$^{154Q/2Q}$ mice (see Figure 4C). This suggests that increased sucrose consumption, unlike other features of the *Atxn1*$^{154Q/2Q}$ model, can be caused by the shorter 78Q polyglutamine tract.

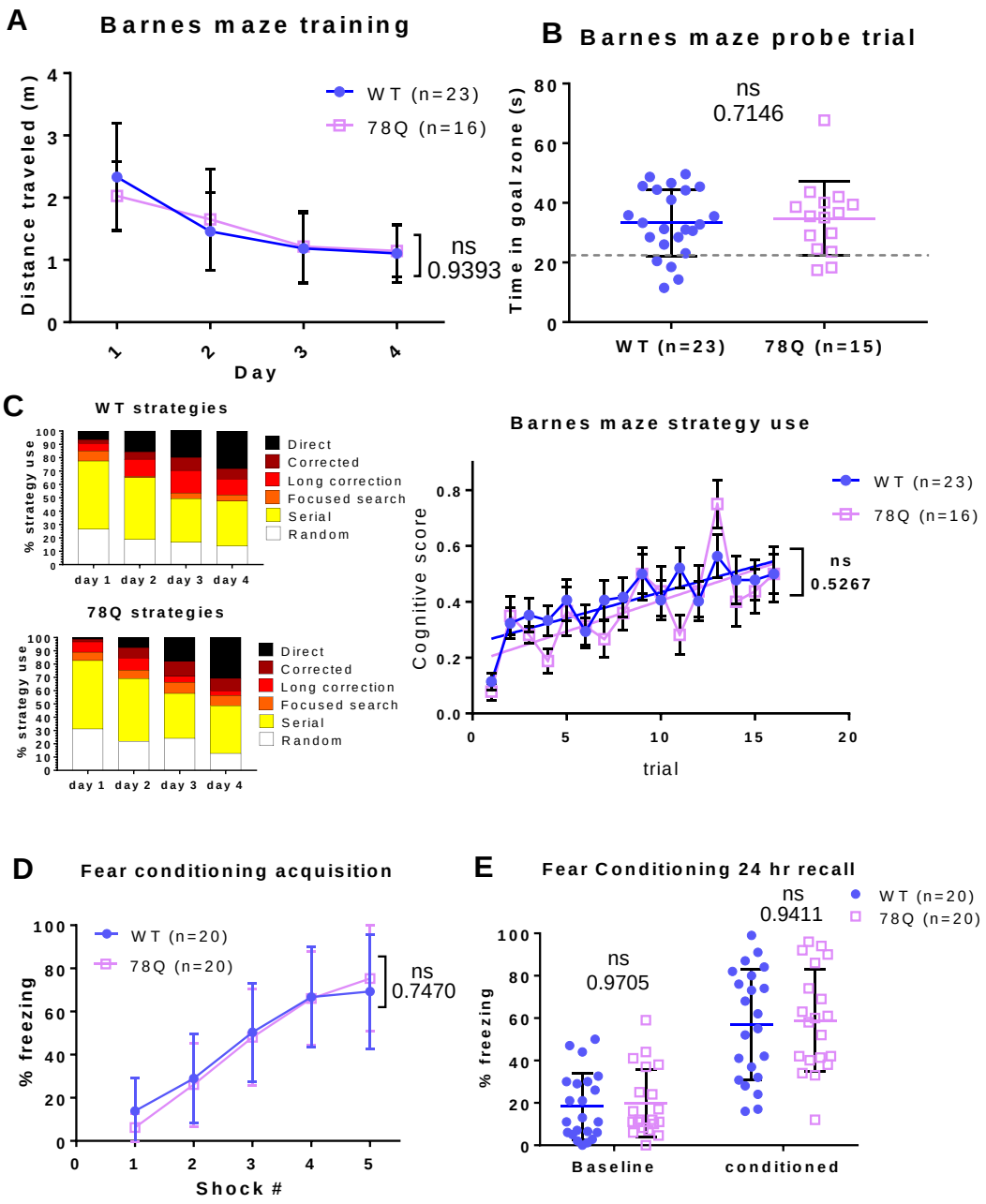

Figure 5. *Atxn1*$^{78Q/2Q}$ mice perform at WT levels on tests of learning and memory. A) Barnes maze average path length on each of the four training days for 9-10 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice (2-way ANOVA). **B)** Barnes maze probe trial (percent time spent in each zone) for 9-10 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice (2-way ANOVA). **C)** Barnes maze search strategy use (left) and corresponding cognitive scores (right) over the four training days in 9-10 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice **D)** Acquisition of freezing responses during context fear conditioning in 10-11 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice, reported as percent freezing during the 60-second interval after each shock (2-way ANOVA). **E)** Fear conditioning 24 hour recall in a baseline context and the conditioned context for 10-11 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice (2-way ANOVA). Data are presented as mean ± SD.)

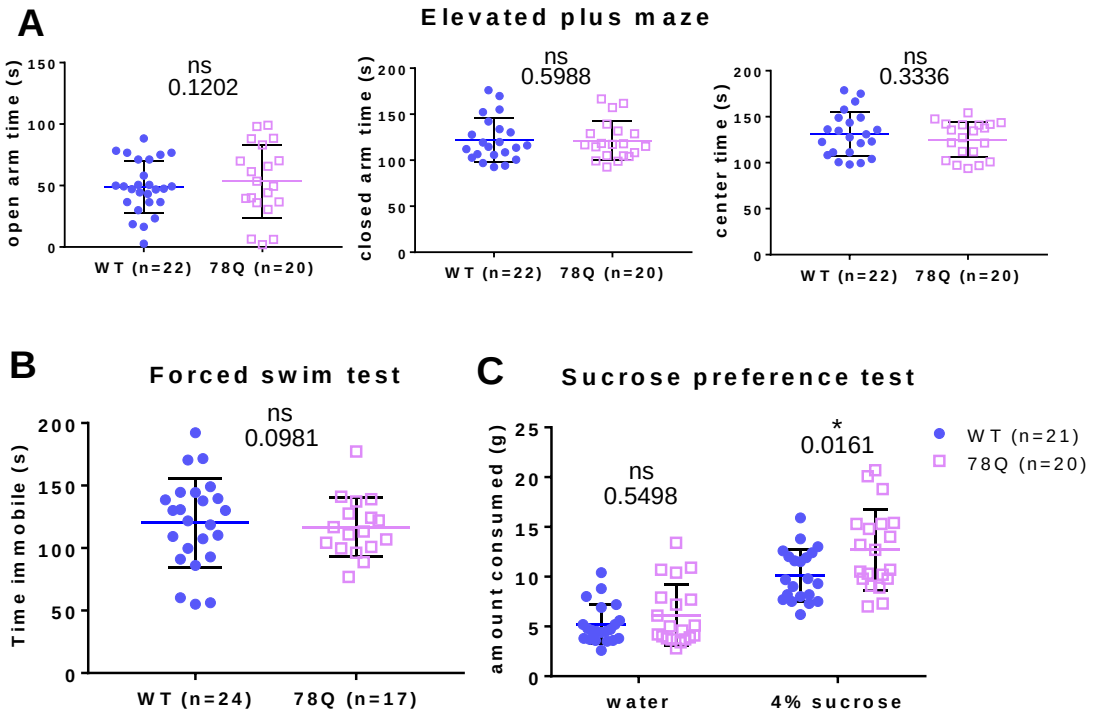

Figure 6. Mood in *Atxn1*$^{78Q/2Q}$ mice. A) Elevated plus maze in 8-9 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice. The time spent in the open arms (left), closed arms (center) and center of the maze (right) is shown (t-test). **B)** Forced swim test in 8-9 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice, reported as time spent immobile in the last 4 minutes of the test (t-test). **C)** Water and sucrose consumption in the sucrose preference test in 11-12 week old WT and *Atxn1*$^{78Q/2Q}$ knockin mice. (2-way ANOVA). Data are presented as mean ± SD.

Chapter 3: Cognition and mood in *PCP2-Atxn1[82Q]* transgenic mice

Introduction

The fact that both *Atxn1*$^{-/-}$ and *Atxn1*$^{154Q/2Q}$ mice perform poorly on tests of cognition strongly suggests that *ATXN1* impacts learning and memory, but provides little insight into how it does so. One possible explanation is that *ATXN1* affects cerebellar function, as it does when causing motor deficits in *Atxn1*$^{154Q/2Q}$ mice and human SCA1 patients. The contribution of cerebellar dysfunction to *ATXN1*-mediated motor deficits in mice has been demonstrated using *Pcp2-ATXN1[82]* transgenic mice, which overexpress polyglutamine-expanded mutant *ATXN1* specifically in cerebellar Purkinje neurons (Burright *et al.* 1995). *Pcp2-ATXN1[82Q]* mice also provide an excellent opportunity to test whether mutant *ATXN1*-induced Purkinje cell dysfunction causes cognitive deficits or mood disturbances in mice; however, we know of no previous study addressing cognition or mood in these mice. We therefore conducted tests of cognition and mood on *Pcp2-ATXN1[82Q]* mice to determine whether *ATXN1* also affects these nonmotor functions by affecting the cerebellum.

Methods

Animals. Pcp2-ATXN1[82Q] transgenic mice (hereafter referred to as *ATXN1[82Q]* mice) were a gift from the laboratory of Dr. Harry Orr. They are from a new transgenic line generated to replace the original B05 line (Burright *et al*. 1995) after the B05 line lost a significant portion of its CAG repeat tract through random mutation. These mice were generated on an FVB background and later backcrossed onto a C57/Bl6

background. All experimental mice tested negative for the homozygous *Pde6* mutation which causes blindness in FVB mice. 8-12 week old male and female *ATXN1[82Q]* mice as well as their wild-type littermates were used.

Behavioral tests and statistical analysis. The Barnes maze, context fear conditioning, elevated plus maze, forced swim, and sucrose preference tests were conducted and analyzed as described above (see Chapter 1), except that t-tests were used in place of one-way ANOVA for comparisons between two groups.

Excluded mice. No mice were excluded from the Barnes maze analysis. For fear conditioning, one *ATXN1[82Q]* mouse clung to the walls and avoided one of the conditioning shocks, two more *ATXN1[82Q]* mice were handled excessively after mistakenly being put in the chambers before the camera was calibrated and being taken back out again before testing, and one WT mouse escaped from the testing rig after conditioning and was excluded from further testing. Three mice, all *ATXN1[82Q]*, fell from the elevated plus maze and were excluded from analysis. One WT mouse was excluded from the forced swim test because the software did not track it properly. On the sucrose preference test, one WT mouse was excluded after its cage flooded overnight from a leaky water bottle.

Results

In order to test the contribution of cerebellar dysfunction to learning and memory deficits in SCA1 model mice, we performed tests of cognition and mood on *ATXN1[82Q]* transgenic mice. These mice overexpress *ATXN1* with 82 CAG repeats selectively in cerebellar Purkinje neurons under the Purkinje cell protein 2 *(Pcp2)* promoter. As with

the *Atxn1*$^{154Q/2Q}$ mice, we tested these mice from 8-12 weeks of age in the hopes of avoiding severe ataxia which could interfere with the interpretation of results.

Figure 7 shows learning and memory results in *ATXN1[82Q]* transgenic mice. On the Barnes maze, *ATXN1[82Q]* mice were largely indistinguishable from wild type in terms of path length over the four training days (no significant effect of genotype [$F_{(1,29)}$ = 1.529 P=0.2261] or genotype x day interaction [$F_{(3,87)}$ = 1.212, P=0.3103] by two-way RM ANOVA, n=15 WT and 16 *ATXN1[82Q]* mice, Figure 7A) and time in the goal zone on the probe trial (WT average 40.55 sec, n=15; ATXN1[82Q] average 37.56 sec, n=16; p=0.4060, t-test, Figure 7B). However, *ATXN1[82Q]* mice appeared to acquire spatial strategies more slowly than WT mice, as evidenced by a significantly lower slope on the regression line of their cognitive score over time (WT: Y = 0.02103*X + 0.1713, *ATXN1[82Q]:* Y = 0.007595*X + 0.166, significantly different slopes, $F_{(1,491)}$=5.586, p=0.0185). This is puzzling given their wild-type-level performance on the probe trial, which indicates that they remember where to find the hole despite apparently not using search strategies that depend on that memory. One possible explanation is that it was relatively common for *ATXN1[82Q]* to find the escape box but continue exploring the maze rather than climbing in, a behavior which was somewhat common among our mice. Trials in which this happened were generally classified as "random" by the strategy analysis software, lowering the average cognitive score without affecting path length (which was measured as the length traveled before the first entry into the goal hole). If this was more common in the *ATXN1[82Q]* mice than in WT, it may explain the apparent difference in strategy use. This could mean that they actually have something other than a

learning deficit, such as a decreased fear of brightly lit spaces (see also the elevated plus maze results below) or subtle motor deficits which make it difficult for them to climb into the escape box without reducing their average walking speed.

In context fear conditioning, *ATXN1[82Q]* mice acquired freezing responses at approximately the same rate as wild type mice during conditioning (no significant effects of genotype [$F_{(1,22)}$ = 0.03942, p=0.8444] or genotype x shock# interaction [$F_{(4, 88)}$=1.023, p=0.4002] by 2-way RM ANOVA, n=12 WT and 12 *ATXN1[82Q]* mice, figure 7D). There was a trend towards *ATXN1[82Q]* mice freezing less than wild type in the conditioned context, but this was not statistically significant (baseline context: WT average 30.08% freezing, *ATXN1[82Q]* average 21.75% freezing; conditioned context: WT average 60.17% freezing, *ATXN1[82Q]* average 45.58% freezing; no significant effect of genotype by 2-way RM ANOVA [$F_{(1,22)}$ = 2.816, P=0.1075], n=12 WT and 12 *ATXN1[82Q]* mice).

Figure 8 shows mood results in *ATXN1[82Q]* mice. The elevated plus maze revealed a striking reduced anxiety phenotype in the *ATXN1[82Q]* mice: they spent significantly more time in the open arms (WT average 30.22 ± 4.745 sec, *ATXN1[82Q]* average 67.99 ± 9.448 sec, p=0.0047, t-test, n=11 WT and 8 *ATXN1[82Q]* mice) and less time in the closed arms (WT average 165.6 ± 5.257 sec, *ATXN1[82Q]* average 126.9 ± 10.58 sec, p=0.0079, t-test, n=11 WT and 8 *ATXN1[82Q]* mice) than wild-type, with some individuals showing little to no preference for the closed arms over the open arms (Figure 8A).

We did not observe any differences between *ATXN1[82Q]* and wild-type mice in either the forced swim test (WT average 137.3 ± 8.064 sec, *ATXN1[82Q]* average 136.5 ± 9.38 sec, p=0.9516, t-test, n=11 WT and 10 *ATXN1[82Q]* mice, Figure 8B) or the sucrose preference test (no significant effect of genotype [$F_{(1,27)}$ = 0.07114, P=0.7917] or genotype x sucrose interaction [$F_{(1,27)}$ = 0.6926, P=0.4126] by 2-way RM ANOVA, n=14 WT and 15 ATXN1[82Q] mice, Figure 8C), suggesting that these mice do not have a strong depressive-like phenotype.

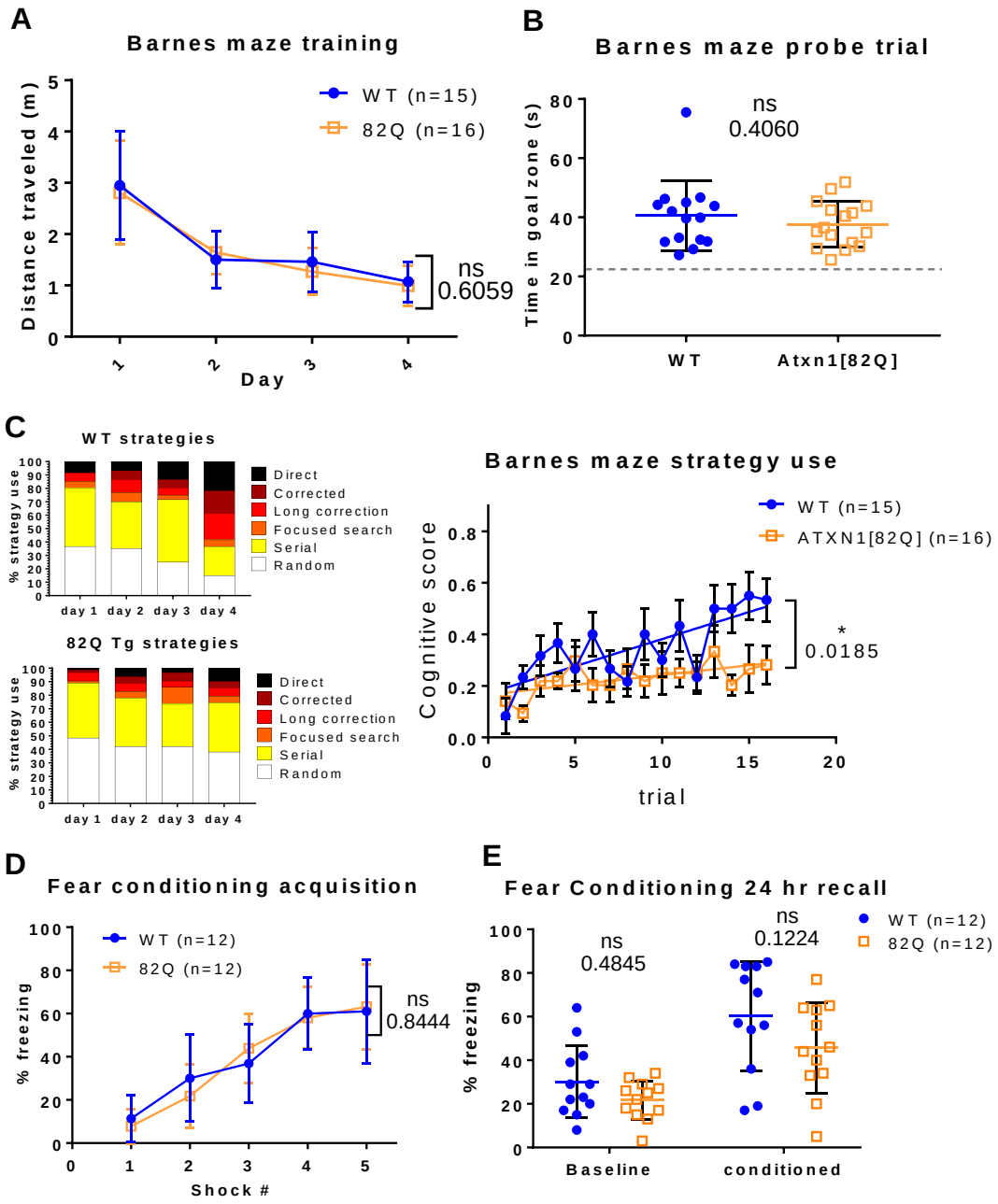

Figure 7. Cognition in *PCP2-ATXN1[82Q]* mice. A) Barnes maze average path length on each of the four training days for 9-10 week old WT and *Pcp2-ATXN1[82Q]* mice (2-way ANOVA). **B)** Barnes maze probe trial (percent time spent in each zone) for 9-10 week old WT and *Pcp2-ATXN1[82Q]* mice (2-way ANOVA). **C)** Barnes maze search strategy use (left) and corresponding cognitive scores (right) over the four training days in 9-10 week old WT and *Pcp2-ATXN1[82Q]* mice. **D)** Acquisition of freezing responses during context fear conditioning in 10-11 week old WT and *Pcp2-ATXN1[82Q]* mice, reported as percent freezing during the 60-second interval after each shock (2-way ANOVA). **E)** Fear conditioning 24 hour recall in a baseline context and the conditioned context for 10-11 week old WT and *Pcp2-ATXN1[82Q]* mice. (2-way ANOVA). Data are presented as mean ± SD.

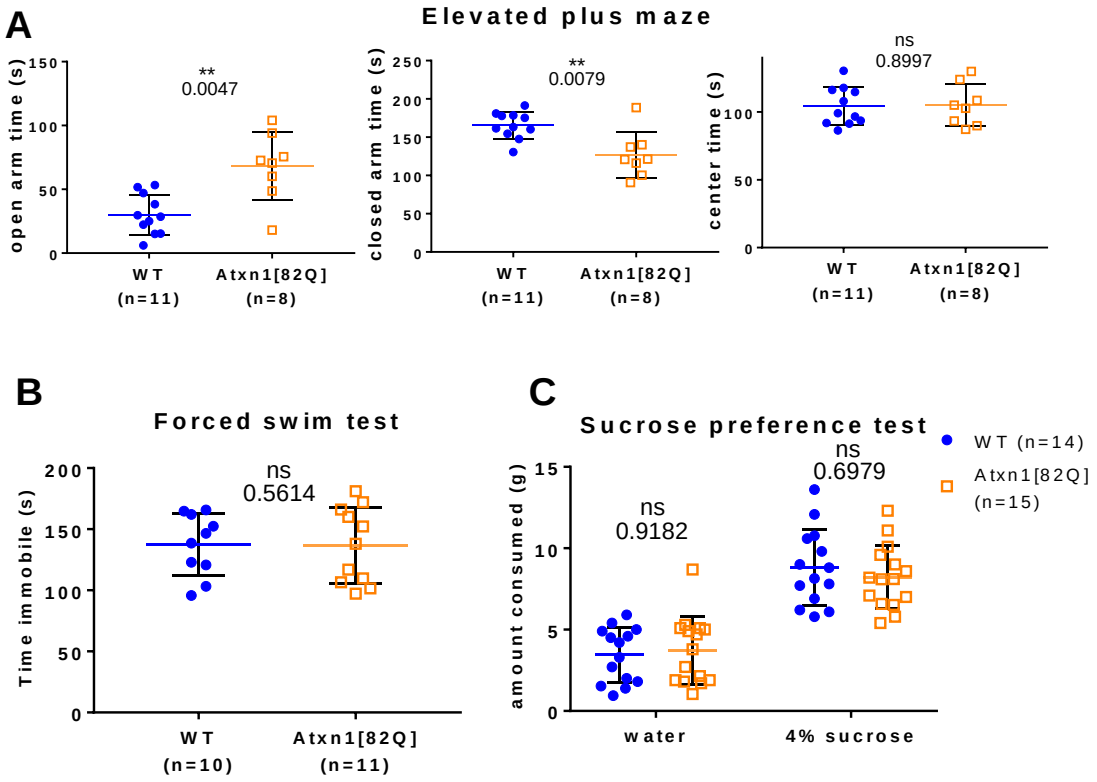

Figure 8. Mood in *PCP2-ATXN1[82Q]* mice. A) Elevated plus maze in 8-9 week old WT and *Pcp2-ATXN1[82Q]* transgenic mice. The time spent in the open arms (left), closed arms (center) and center of the maze (right) is shown (t-test). **B)** Forced swim test in 8-9 week old WT and *Pcp2-ATXN1[82Q]* transgenic mice, reported as time spent immobile in the last 4 minutes of the test (t-test). **C)** Water and sucrose consumption in the sucrose preference test in 11-12 week old WT and *Pcp2-ATXN1[82Q]* transgenic mice (2-way ANOVA). Data are presented as mean ± SD.

Chapter 4: *ATXN1* knockout reduces hippocampal neurogenesis

Note regarding ownership and author contributions

This chapter includes figures and text originally published in *Neuroscience* (Asher *et al.* 2016) and reproduced here with permission from the journal. The methods and results sections were written jointly by me, Andrea Johnson, and Marija Cvetanovic, based on data collected by me (neurosphere culture, transfection, and BrdU assays; BrdU immunohistochemistry; Western blotting), Andrea Johnson (DAOY cell culture; cell cycle assays; RT-qPCR, Western blotting), Bojana Zecevic (immunohistochemistry) and David Pease (immunohistochemistry). The introduction has been rewritten by me for this thesis.

Introduction

In addition to characterizing the behavioral changes in ataxin-1 mutant mouse strains, we are interested in finding the cellular and molecular mechanisms of these changes—this will contribute to our understanding of ataxin-1's endogenous function and could inform future studies aimed at understanding, treating, or preventing cognitive changes in humans with SCA1. However, given that the cerebellum-specific *Pcp2-ATXN1[82Q]* mutant showed little to no cognitive deficits on the tasks we used, it is likely that areas other than the cerebellum make the largest contributions to ataxin-1's role in cognition. We therefore decided to examine the role of *ATXN1* in the hippocampus, which is well known for its major contributions to learning and memory in both humans and rodents

(Scoville and Milner 1957), and is thought to be crucial for both spatial navigation (O'Keefe and Dostrovsky 1971) and context fear conditioning (Shima *et al.* 2013).

The hippocampus performs many functions related to learning and memory. Our results focus on the process of hippocampal neurogenesis, or the generation of adult-born hippocampal granule neurons. Hippocampal neurogenesis continues throughout adult life. It depends on a pool of neural precursor cells (NPCs) in the subgranular zone of the hippocampal dentate gyrus, which consists of slowly dividing stem cells and more quickly dividing intermediate progenitor cells. Most of the dentate gyrus stem cells are quiescent at any given time, but they can enter the cell cycle and divide to give rise to intermediate progenitor cells. Intermediate progenitor cells undergo further divisions, differentiate into young neurons, and migrate a short distance into the granule cell layer, where they integrate into the neural circuitry (Deng *et al.* 2009; Christian, Song, and Ming 2014). For some time after being integrated, young adult-born neurons have unique circuit properties (Doetsch and Hen 2005). This allows them to contribute in unique ways to pattern separation, or distinguishing between similar inputs, which may be important for avoiding interference between new and old memories (Nakashiba *et al.* 2012; Johnston *et al.* 2015).

The rate at which new neurons are generated has been linked to learning and memory in numerous previous studies (Jessberger *et al.* 2009; Ko *et al.* 2009; Braun and Jessberger 2014; Shors *et al.* 2001; Dupret *et al.* 2008; Sahay *et al.* 2011). Hippocampal neurogenesis is also reduced in a number of disease states where cognition is impaired, including Alzheimer's disease (Verret *et al.* 2007), Parkinson's disease (Winner *et al.*

2004), and Huntington's disease (Fedele *et al.* 2011). To begin testing whether this is also true in SCA1, we investigated the role of ataxin-1 in regulating adult hippocampal neurogenesis.

Methods

Animals. *Atxn1*$^{-/-}$ mice were generated as described (Matilla *et al.* 1998). Originally generated on a C57BL/6J–129/SvEv mixed background, these mice were backcrossed for more than ten generations with FVB mice to avoid confounding effects of genetic background. Because our previous studies have detected no sex-specific effects, we have used an equal mix of animals of both sexes for our experiments. All animal experiments were performed in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and the University of Minnesota Institutional Animal Care and Use Committee.

NPC culture. Hippocampal precursor cells were isolated from hippocampi of one-month-old mice according to published isolation protocols (Bonaguidi *et al.* 2008). Briefly, dissected hippocampi of *Atxn1*$^{-/-}$ mice and their wild-type littermates were incubated in 0.1% trypsin for 7 min at 37°C. Trypsin inhibitor (Sigma) was added to stop the digestion, and the cell suspension was passed through a 70-μm cell strainer. Cells were grown in neurosphere medium (DMEM/F12 with l-glutamine (Gibco) containing penicillin/streptomycin with l-glutamine (Gibco), heparin (Sigma), N2 (Gibco), B27 (Gibco), 10 ng/mL human recombinant fibroblast growth factor (FGF, BD), 20 ng/mL

human recombinant epidermal growth factor (EGF, BD) and 250 ng/ml noggin (R&D)) at 37°C in non-adherent 24-well plates.

Nucleofector4D transfection of neurospheres. Hippocampal precursor cells were isolated from one-month-old wild-type (FVB) or *Atxn1*$^{-/-}$ mice and grown as neurospheres. After being expanded *in vitro* for 4-7 passages, neurospheres were dissociated into a single-cell suspension using trypsin (0.05%) and mechanical trituration. Dissociated cells were transfected using the Lonza 4-D Nucleofector X Unit (Lonza) according to the manufacturer's instructions. Briefly, 100,000–500,000 cells per condition were resuspended in 20 μl P3 solution (Lonza) and 1 μg of plasmid DNA (pEGFP-C2 with either ATXN1[82Q] or ATXN1[30Q] inserted into the EcoRI/SalI sites to make ATXN1 with GFP fused to the C-terminal, or pEGFP-C2 alone as a control). The cell suspension was subjected to pulse code CU-110 and allowed to sit for 10 min at room temperature before being plated in neurosphere growth medium at a density of approximately 50,000 cells/mL.

5-bromo-2'-deoxyuridine (BrdU) administration and immunohistochemistry. BrdU (Sigma–Aldrich) was prepared at 20 mg/mL in sterile saline and injected intraperitoneally at a dose of 100 mg/kg every 12 h for three doses. Mice were euthanized 48 h or one month after injection. Brains were harvested and post-fixed by incubation in 4% paraformaldehyde overnight, and then incubated in 30% sucrose until saturation. 40-μm-thick sections were cut using a cryostat (Leica 1900). Antigen retrieval was performed with 10 mM sodium citrate (pH 6) for 25 min at 100°C. The sections were

incubated for 1 h in blocking buffer (3% Normal Donkey Serum in PBST (pH 7.5, 50 mM Tris-HCl, 145.5 mM NaCl, 1 % TritonX-100)) followed by overnight incubation at 4°C in blocking buffer containing primary antibody (anti-NeuN (goat, Santa Cruz #SC8066), anti-ataxin1 (rabbit 11750, gift from Dr. Harry Orr), anti-Tbr2 (chicken, Millipore #AB15894) and anti-Sox2 (goat, Santa Cruz #sc-17320)). After being exposed to secondary anti-goat or anti-rabbit Alexa 594 conjugated antibody (Life Technologies), samples were fixed with 4% paraformaldehyde and treated with 5 M HCl in 0.1% TritonX-100 for 15 min to expose BrdU binding sites. After 5 washes in PBS, samples were incubated overnight in anti-BrdU antibody (rat, Bio-Rad #MCA2060) followed by secondary anti-rat Alexa 488 antibody. Samples were mounted using anti-fade mounting medium with DAPI (Vectashield, Vector) and analyzed using an Olympus FV1000 confocal microscope.

Western blot analysis. NPCs derived from *Atxn1*$^{-/-}$ mice and wild-type littermates were lysed in RIPA lysis buffer (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1% sodium deoxycholate, 1% NP-40, 0.2% SDS, phosphatase inhibitor (Sigma) and protease inhibitor cocktail (Roche)). Proteins were separated on a 12% SDS–PAGE gel and transferred onto a nitrocellulose membrane. The following primary antibodies were used: anti-ATXN1 (rabbit 11750 and 11NQ, gifts from Dr. Harry Orr's laboratory), anti-cyclin D1 (mouse, Santa Cruz #20044; rabbit, Abcam #ab134175), and alpha-tubulin (mouse, Sigma). Signal from secondary antibodies linked to horseradish peroxidase (HRP) (GE Healthcare) was detected using Amersham ECL Western Blotting Detection Reagent (GE

Healthcare) and an ImageQuant LAS 4000 imager (GE Healthcare); protein levels were quantified using ImageQuant (GE healthcare) software.

In vitro BrdU proliferation assay. A BrdU proliferation assay was performed using the BrdU Cell Proliferation Assay Kit (Cell Signaling) according to the manufacturer's instructions. Neurospheres were trypsinized and mechanically dissociated into single-cell suspensions, and 10,000 NPCs were plated per well in non-adherent 96-well plates. BrdU solution was added at the time of cell plating and cells were allowed to grow for 24 h. To assay BrdU incorporation, cells were fixed and incubated with primary BrdU antibody for 1 h at RT, followed by incubation with HRP-conjugated secondary antibody for 30 min. TMB substrate was added to the wells and absorbance was read at 450 nm using a dual-wavelength spectrophotometer (SPECTRAmax). Each experimental condition included 4–5 technical replicates and the experiment was performed on four independent biological isolates.

Secondary neurosphere formation assay. Early passages (P1-P7) of NPCs were plated in non-adherent 96-well plates (BD 351172) at a density of 1000 cells/well, 8–12 wells per condition. After 7 days, neurospheres, defined as clumps of more than 16 cells, were imaged; neurospheres were counted and their diameter was measured using ImageJ (NIH). Neurospheres that had flat cells with processes spreading out of the spheres were counted as differentiating.

Statistical analysis. Data are presented as mean ± SEM. Statistical analysis was performed by Student's *t*-test. Results are considered statistically significant when $P < 0.05$.

Results

Ataxin-1 is expressed in hippocampal NPCs

In light of the evidence linking *ATXN1* to hippocampal learning in mice, we examined the expression pattern of *ATXN1* in the hippocampus. Consistent with published data, we found that *ATXN1* was strongly expressed in granule neurons of the dentate gyrus (Banfi *et al.* 1996; Servadio *et al.* 1995). Additionally, we found that *ATXN1* was expressed in NPCs; here we use the term *neural precursor cells* to encompass neural stem cells (expressing sex determining region Y-box 2 (Sox2)) and neural progenitor cells (expressing T-box brain gene 2 (Tbr2)) (Bonaguidi *et al*. 2011; Hodge *et al*. 2012) (Figure 9A). Western blot analysis of lysates prepared from isolated precursor cells grown *in vitro* as neurospheres confirmed expression of *ATXN1* in these cells (Figure 9B).

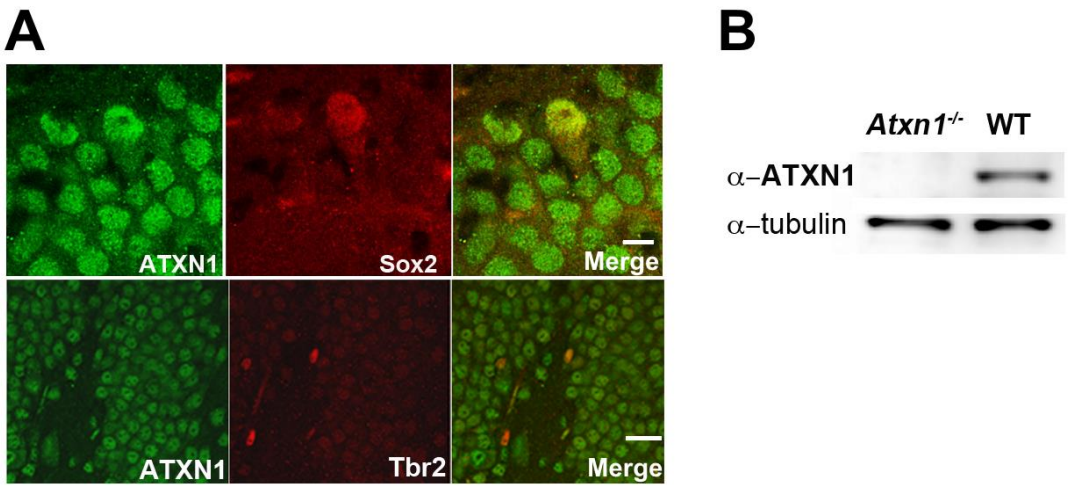

Figure 9. ***ATXN1*** **is expressed in dentate gyrus. A)** Confocal images of hippocampal dentate gyrus co-stained for *ATXN1* (green) and markers of neural precursor cells: (Sox2) (red, top right panel) and (Tbr2) (red, left and bottom right panel). Scale bars=50, 10, and 20 µm. **B)** Western blot of lysates prepared from neural precursor cells derived from *Atxn1*$^{-/-}$ and wild-type mice, labeled with anti-ataxin-1 and anti-alpha-tubulin antibodies.

Hippocampal proliferation is progressively decreased in Atxn1$^{-/-}$ mice

Expression of *ATXN1* in precursor cells of the dentate gyrus suggests a potential function in adult hippocampal neurogenesis. To investigate this, we assessed proliferation in the dentate gyrus of *Atxn1*$^{-/-}$ mice with intraperitoneal injection of BrdU, a thymidine analog that is incorporated into the DNA during cell division. BrdU-immunoreactive cells were quantified in the dentate gyrus of the hippocampus by immunohistochemistry. One-month-old *Atxn1*$^{-/-}$ mice already had 25% fewer BrdU^{+} cells in the dentate gyrus than wild-type controls (Figure 10A). This deficit worsened with age; at one month of age (postnatal day 30 (P30)) there was a 25% decrease (P = 0.0459), at two months (P60) a 37% decrease (P = 0.0146), and at four months (P120) a 72% decrease (P = 0.0002) in the number of proliferating cells in the dentate gyri of *Atxn1*$^{-/-}$ mice compared to their wild-type littermates (Figure 10B). Co-labeling of BrdU-positive cells with markers of neural stem cells (glial fibrillary acidic protein (GFAP) and nestin) demonstrated that neural stem cells proliferate less in *Atxn1*$^{-/-}$ mice (data not shown). These data suggest that *ATXN1* is required to maintain proliferation of hippocampal neural precursors.

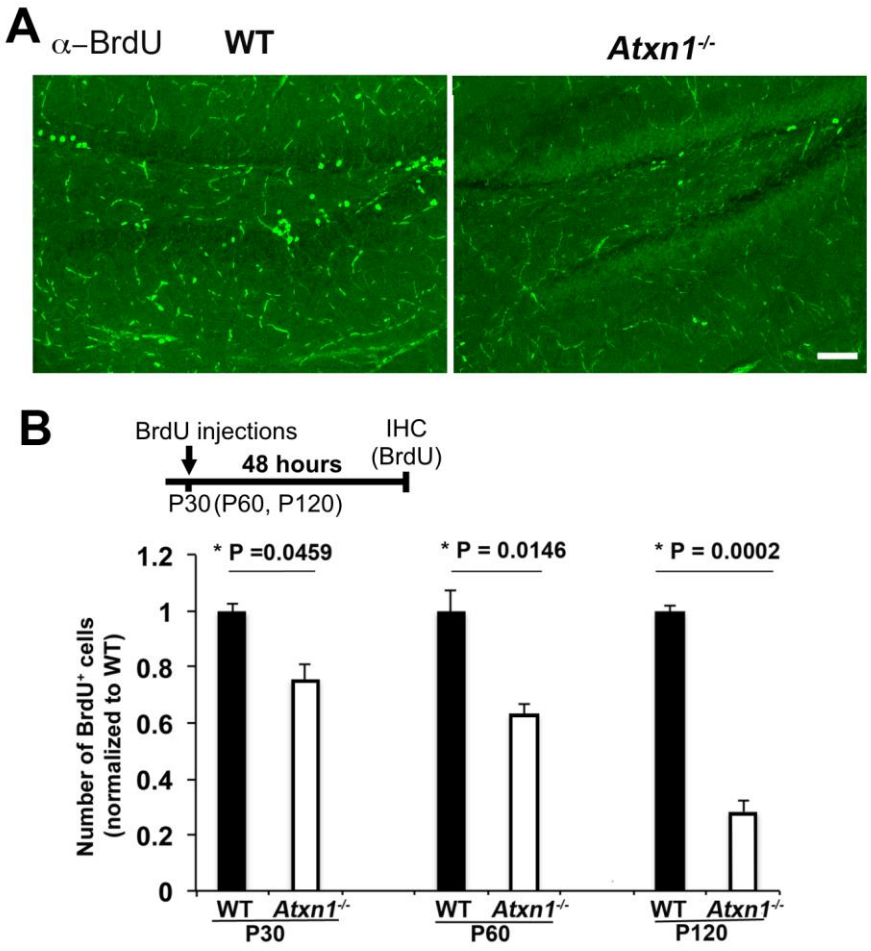

Figure 10. Hippocampal proliferation is decreased in *Atxn1*$^{-/-}$ mice. A) Representative image of BrdU staining in *Atxn1*$^{-/-}$ mice and wild-type littermates. Scale bar=40 µm. **B)** The number of BrdU^{+} cells per dentate gyrus 48 h after the last BrdU injection in P30, P60 and P120 *Atxn1*$^{-/-}$ mice normalized to wild-type mice (Student's t-test, P=0.0459, P=0.0146, and P=0.0002 respectively). N=3 mice per group. Error bars=SEM.

Decreased proliferation in the dentate gyrus of Atxn1$^{-/-}$ mice results in a reduced number of newly formed hippocampal neurons

To determine whether the reduction in hippocampal proliferation ultimately affected the number of newly formed neurons—the final output of hippocampal neurogenesis—we birth-dated newborn neurons with intraperitoneal injections of BrdU and analyzed the brains one month later for co-expression of BrdU and the mature neuronal marker NeuN (Figure 11A). At both ages examined (one and two months at time of injection) *Atxn1*$^{-/-}$ mice had fewer newly generated BrdU^{+}NeuN^{+} neurons in the dentate gyrus than their age-matched wild-type littermates. Furthermore, this reduction in newly generated hippocampal neurons worsened with age (P30: 25% decrease, P = 0.037, P60 75% decrease, P = 0.0004, Figure 11B) suggesting that lack of *ATXN1* progressively reduces hippocampal neurogenesis.

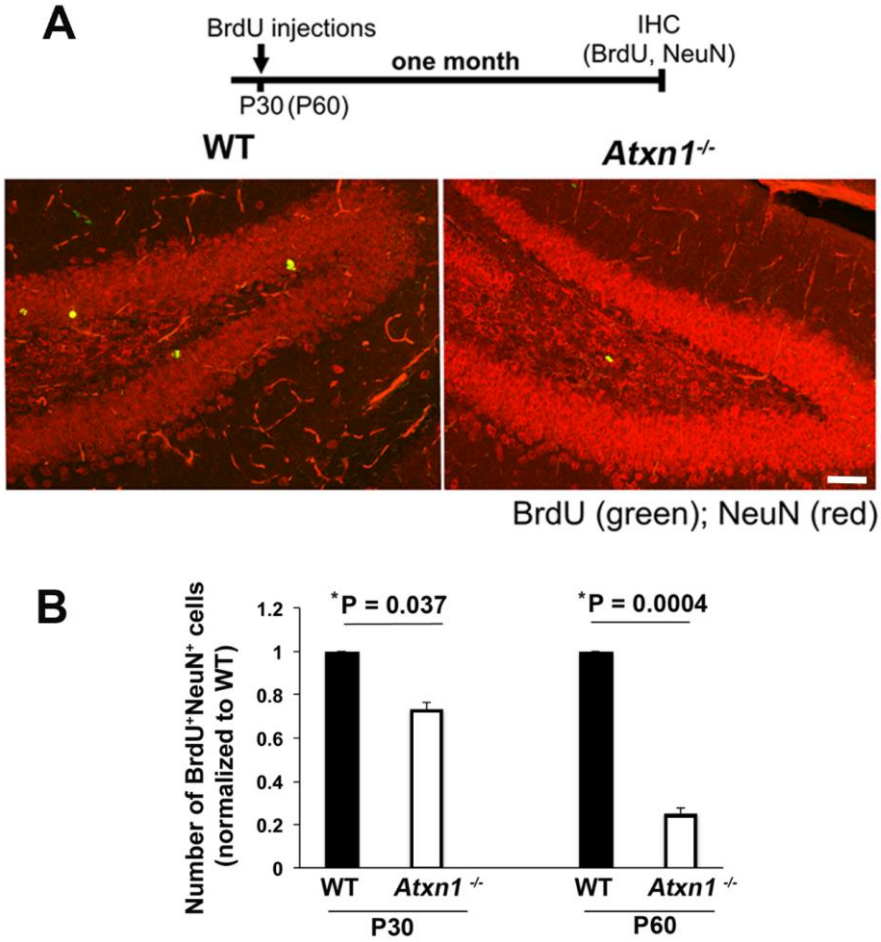

Figure 11. Hippocampal neurogenesis is decreased in Atxn1$^{-/-}$ mice. A) Representative image of BrdU (isolated green nuclei) and NeuN (tightly-packed red nuclei) co-staining in *Atxn1*$^{-/-}$ mice and wild-type littermates. Scale bar=40 µm. **B)** The number of newborn mature neurons (BrdU^{+}NeuN^{+}) per dentate gyrus one month after BrdU injection into P30 and P60 Atxn1$^{-/-}$ mice, normalized to wild-type littermates (Student's t-test, P=0.037 and P=0.004 respectively). N=3 mice per group. Error bars=SEM.

ATXN1 regulates hippocampal proliferation in a cell-autonomous manner

Proliferation of NPCs is determined by the interaction of extrinsic and intrinsic factors (Faigle and Song 2013). For example, several factors are known to be released within the neurogenic niche from mature neurons to regulate the proliferation of NPCs (Ma *et al.* 2009). Because *ATXN1* is expressed both in neural precursors and in mature granule neurons, loss of *ATXN1* could alter proliferation of NPCs either in a cell-autonomous manner and/or by environmental signals such as those from mature neurons. To test whether *ATXN1* can regulate proliferation of cells in a cell-autonomous manner, we isolated hippocampal precursors from *Atxn1*$^{-/-}$ mice and cultured them *in vitro* as neurospheres (Figure 12A). An *in vitro* BrdU assay revealed decreased cell proliferation of NPCs derived from P30 *Atxn1*$^{-/-}$ mice (Figure 12B, 21% decrease, $P = 0.0148$, $N = 4$ independent isolates). In a secondary neurosphere formation assay, the decreased proliferation potential of *Atxn1*$^{-/-}$ NPCs was evident by smaller neurospheres (Figure 12C, 23% decrease in diameter, $P = 0.0148$, $N = 5$). This decreased proliferation potential was accompanied by a greater degree of differentiation, as defined by the presence of flat cells extending processes beyond the boundaries of the spheres (4.9% of wild-type spheres and 24.67% of *Atxn1*$^{-/-}$ spheres, $P = 0.038$, $N = 5$, Figure 12D). This suggests that *ATXN1* regulates proliferation of NPCs in a cell-autonomous manner, although it does not rule out the possibility that *ATXN1* also modulates neurogenesis indirectly by affecting granule neurons or other cells of the neurogenic niche (Ma *et al*. 2009; Song *et al.* 2012). NPCs isolated from two-month-old *Atxn1*$^{-/-}$ mice were also significantly impaired in the BrdU incorporation assay (50% decrease in BrdU incorporation, $N = 3$

independent isolates, P = 0.034, data not shown). The reduction in proliferation was greater in NPCs isolated from two-month-old *Atxn1*$^{-/-}$ mice than in those isolated from one-month-old *Atxn1*$^{-/-}$ mice (50% at two months vs 21% at one month); this suggests a progressive decrease in the intrinsic proliferative potential of *Atxn1*$^{-/-}$ hippocampal NPCs, in agreement with our *in vivo* results (Figures 10 and 11). Interestingly, half of our attempts to grow neurospheres from two-month-old *Atxn1*$^{-/-}$ mice were unsuccessful. In the remaining half of isolates, several passages were required to grow enough *Atxn1*$^{-/-}$ cells to perform a proliferation assay, compared to just one passage in cells isolated from wild-type littermates. Finally, expression of human wild-type ATXN1[30Q] in *Atxn1*$^{-/-}$ neurospheres rescued the proliferation deficit in the BrdU assay (Figure 12E, ~20% increase compared to GFP transfected *Atxn1*$^{-/-}$ control, N = 3, P = 0.041), but did not significantly increase the diameter of *Atxn1*$^{-/-}$ neurospheres or prevent their differentiation (data not shown).

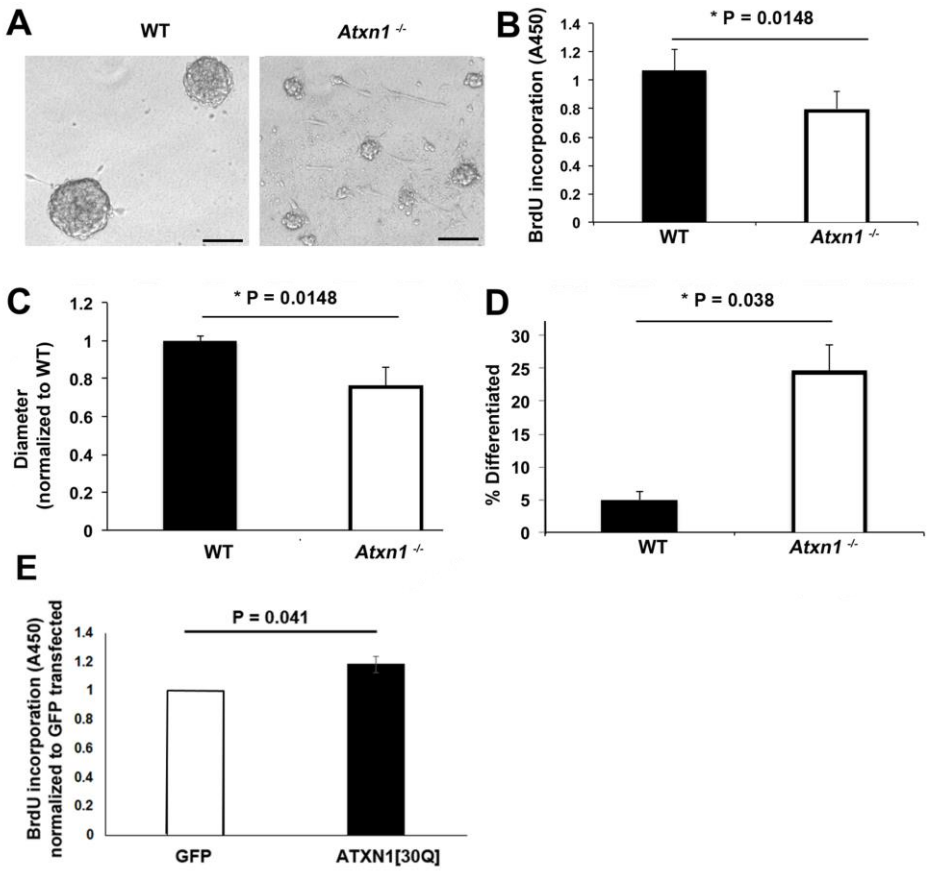

Figure 12. Neural precursor cells isolated from *Atxn1*$^{-/-}$ hippocampus proliferate less than wild-type cells *in vitro*. Neural precursor cells isolated from P30 *Atxn1*$^{-/-}$ mice and their wild-type littermates were grown *in vitro*. **A)** Bright-field image of WT and *Atxn1*$^{-/-}$ neurospheres. Scale bar=100 µm. **B)** An *in vitro* BrdU assay demonstrates decreased proliferation of *Atxn1*$^{-/-}$ precursors (Student's t-test, P=0.0148). **C) and D)** Colonies formed from *Atxn1*$^{-/-}$ cells have decreased diameter compared to wild type **(C)** (Student's t-test, P=0.0148), as well as a higher degree of differentiation **(D)**, defined by the presence of flat cells with processes extending out of the spheres (Student's t-test, P=0.038). N=5 independent cultures of neural precursor cells. **E)** Transient transfection of wild-type ATXN1[30Q] increased BrdU incorporation of *Atxn1*$^{-/-}$ precursors. N=3 independent transfections. (Student's t-test, P=0.041). Error bars=SEM.

To test whether the SCA1-causing polyglutamine expansion in ATXN1 also affects proliferation of NPCs, we used a Nucleofector 4D system to transiently transfect wild-type neurospheres with plasmid vectors expressing GFP-tagged human mutant ATXN1[82Q], wild-type ATXN1[30Q] or GFP as a control. Expression of mutant ATXN1[82Q] significantly decreased BrdU incorporation in wild-type neurospheres (~40% decrease compared to control GFP-transfected cells, N = 6 independent transfections, P = 0.0168, Figure 13A). Expression of wild-type ATXN1[30Q] caused a trend toward a decrease in BrdU incorporation (~20% compared to GFP control, N = 3, P = 0.055, Figure 13A). In a secondary neurosphere formation assay, ATXN1[82Q]-expressing NPCs formed smaller neurospheres than GFP controls (Figure 13B, ~12% decrease in diameter, N = 3, P = 0.024), with a greater degree of differentiation (Figure 13C, 8.2% of GFP controls, 7.2% of ATXN1[30Q] transfected, and 12.4% of *ATXN1*[82Q] transfected spheres, N = 3, P = 0.031 comparing ATXN1[82Q] to GFP controls). Expression of wild-type ATXN1[30Q] did not affect the size or differentiation of neurospheres (Figure 13B, C). This data suggests that both loss of ataxin-1 and the polyglutamine expansion in ATXN1 reduce proliferation of NPCs.

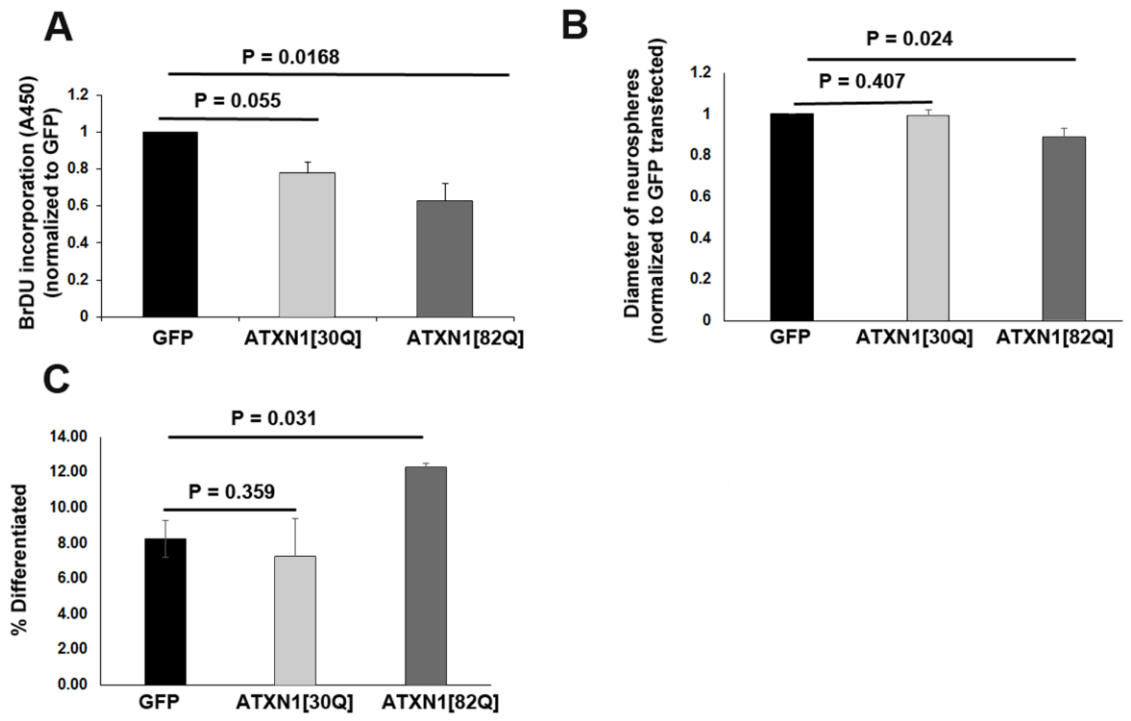

Figure 13. Expression of mutant ATXN1[82Q] reduces proliferation of wild-type neural precursor cells. Neural precursor cells isolated from wild-type mice were transfected with plasmid vectors expressing mutant ATXN1[82Q], wild-type ATXN1[30Q] or control GFP. **A)** An *in vitro* BrdU assay demonstrates decreased proliferation upon ATXN1[82Q] expression compared to GFP controls. ATXN1[30Q] also showed a trend toward decrease. (Student's t-test, P=0.0168 and P=0.055 for ATXN1[82Q] and ATXN1[30Q] respectively, N=6 or 3 independent transfections.) **B) and C)** Colonies formed from ATXN1[82Q]-transfected cells have decreased diameter (**B**, P=0.024) and higher degree of differentiation than wild type (**C**, P=0.031). N=4, 4, 2 for GFP, ATXN1[30Q] and ATXN1[82Q] transfections respectively. Error bars=SEM.

Discussion

Table 1. Summary of results

Strain	Barnes Maze	Fear conditioning	EPM open arm time	FST time immobile	Sucrose preference	Hippocampal Neurogenesis
Atxn1$^{-/-}$	↓	↓	↑	↓	n.s.	↓
Atxn1$^{+/-}$	n.s.	n.s.	n.s.	n.s.	n.s.	
Atxn1$^{154Q/2Q}$	↓	↓	↓	n.s.	↑	↓[1]
Atxn1$^{78Q/2Q}$	n.s.	n.s.	n.s.	n.s.	↑	
Pcp2-ATXN1[82Q]	n.s.[2]	n.s.	↑	n.s.	n.s.	

Table 1. A summary of the major findings presented in chapters 1-4.

Blank cells = not tested.
n.s. = no significant difference between the listed strain and wild-type.
Down arrows = less time in goal zone (Barnes maze probe trial), less time freezing (fear conditioning 24-hour recall), less time in open arms and/or more time in closed arms (Elevated plus maze), less time spent immobile (forced swim test), decreased sucrose consumption (sucrose preference test), or reduced hippocampal neurogenesis.
Up arrows = increased time in the open arms (elevated plus maze) or increased sucrose consumption (sucrose preference test).

1 Cvetanovic, Hu, and Opal 2016. All other cells show results presented in this thesis.
2 No significant difference in performance on the probe trial, but see chapter 3 for a discussion of differences in search behavior during the training trials.

SCA1 patients often experience cognitive deficits and mood alterations, but the root causes of these features of the disease have not been studied extensively, despite the availability of a variety of informative *ATXN1* mutant mouse models. In order to better determine the role of *ATXN1* in cognition and mood, we subjected several mouse strains with modifications in *ATXN1* to a battery of behavioral tests. These included *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice to test haploinsufficiency and loss of function of *ATXN1*, *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ mice to test the effects of polyglutamine expansions of different lengths, and *Pcp2-ATXN1[82Q]* mice to test the contribution of Purkinje cell dysfunction. The results are summarized in Table 1 on the previous page. This is the first time that these strains of mice have all been subjected to the same tests of mood and cognition in the same laboratory, allowing for better comparisons than ever before.

We tested learning and memory in these mice using the Barnes maze and context fear conditioning. We found that *Atxn1*$^{-/-}$ and *Atxn1*$^{154Q/2Q}$ mice were both deficient in recall on these tests, in keeping with previous studies showing deficits on the Morris water maze in these mice (Matilla *et al.* 1998; Watase *et al.* 2002). For the *Atxn1*$^{154Q/2Q}$ mice, the deficits in the Barnes maze probe trial may be due to an issue with acquiring the task, as they failed to develop spatial search strategies during training. We saw no cognitive deficits in *Atxn1*$^{78Q/2Q}$ knockin mice. This suggests that, similarly to motor deficits, cognitive deficits only arise in knockin mice with longer polyglutamine expansions than those seen in human patients.

The presence of learning and memory deficits in both *Atxn1*$^{-/-}$ and *Atxn1*$^{154Q/2Q}$ mice suggests that these deficits are at least partially due to loss of ataxin-1 function rather

than a toxic gain of function in the 154Q allele. Importantly, however, heterozygous *ATXN1* knockout mice performed normally on both Barnes maze and fear conditioning. Even a complete loss of function in the 154Q allele would be similar to a heterozygous knockout. Thus, the 154Q allele may function as a dominant negative, interfering with the function of the wild-type allele. This is further supported by the fact that the Barnes maze deficits during training were more severe in the $Atxn1^{154Q/2Q}$ mice than in the $Atxn1^{-/-}$ mice.

The fact that heterozygous knockout mice perform normally suggests that a 50% decrease in ataxin-1 levels, such as that seen in experimental treatments aimed at reducing ataxin-1 levels, would not cause cognitive side effects in wild-type mice. However, it will be important to determine whether this is true in mice with one allele expressing *ATXN1* with 78Q or 154Q, which are more similar to SCA1 patients and would be starting with some loss-of-function or dominant negative function before treatment. Experimentally reducing ataxin-1 levels in these mice will produce some combination of desirable effects (reducing any dominant-negative effects of the mutant allele) and undesirable effects (further knocking down the wild-type allele) on cognition. It may therefore be advisable to include tests of learning and memory when assessing the effects of preclinical treatments which reduce ataxin-1 levels.

We did not see a significant deficit in performance on the Barnes maze or context fear conditioning in *Pcp2-ATXN1[82Q]* mice, suggesting that *ATXN1* exerts its effects on cognition outside the cerebellar cortex, in contrast to its motor effects which are cerebellum-dependent (Burright *et al.* 1995). However, these mice did show unusual

behavior during training. They acquired spatial strategies slowly compared to wild-type mice. This may have been due to mice continuing to explore the maze despite having found the escape hole, which may indicate changes in their response to the open field or their ability to physically enter the hole. These changes could have interfered with the test, but further analysis is needed to quantify this behavior and determine whether there is a difference between genotypes. In addition, these more hippocampus-dependent tests may also fail to detect cerebellar cognitive deficits, which in humans are often related to executive function and working memory (for review see Koziol *et al*. 2014) rather than episodic memory, which would be more dependent on the hippocampus. Any future studies on cognition in these mice should therefore include tests of motor ability, such as the rotarod, beam cross, or Erasmus ladder, and tests of working memory, such as the alternating T-maze.

Our mood tests focused on depression- and anxiety-like phenotypes. Despite the high prevalence of depression in SCA1 patients, we saw no evidence of a depression-like phenotype in any of our strains of mice. We did, however, see phenotypes in some strains which are not traditionally associated with a depression- or anxiety-like phenotype but which still may point to underlying changes in mood or motivation. These included the reduced immobility time on the forced swim test in *Atxn1*$^{-/-}$ mice, the increased sucrose preference in *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ mice, and the increased time in the open arms of the elevated plus maze in *Atxn1*$^{-/-}$ and *ATXN1[82Q]* mice.

Reduced immobility on the forced swim test is most often observed after treatment with antidepressants, and in juvenile rats this can remain long after treatment is discontinued

(Iñiguez, Warren, and Bolaños-Guzmán 2010). However, this does not necessarily mean that removing ataxin-1 has an "antidepressant" effect. The forced swim test ultimately measures the preference for a passive coping strategy (floating) over a more active escape strategy (swimming) when faced with an inescapable adverse situation (Commons *et al*. 2017). Coping strategy can be affected by both environment (for example, chronic stress leading to a more passive strategy) and innate differences. A variety of poorly understood factors are involved in innate differences in immobility between strains. Our data suggest that *ATXN1* may be among these factors.

Interestingly, some genetic models of autism show reduced immobility in the forced swim test, though others may show increased immobility (Commons *et al*. 2017). This could be an interesting avenue of investigation given that humans lacking *ATXN1* develop autism spectrum disorders (Celestino-Soper *et al.* 2012). In addition, when *CIC*, a major binding partner of *ATXN1*, is deleted from the hypothalamus and lateral amygdala using *Otp-cre*, mice show abnormal social behavior (Lu *et al.* 2017). Two humans with mutations in *CIC* also showed autism spectrum disorders, although the sample size was small (Lu *et al.* 2017). However, much more work would need to be done on social cognition in *Atxn1*$^{-/-}$ mice to determine whether a connection to autism-like behavior might exist.

We attempted to investigate multiple aspects of a possible depressive-like phenotype by using a second test, the sucrose preference test, which measures anhedonia. However, none of our mice showed the decreased sucrose preference traditionally associated with a depressive-like phenotype. We saw *increased* sucrose preference in both *Atxn1*$^{154Q/2Q}$ and

Atxn1$^{78Q/2Q}$ mice, suggesting that the 78Q polyglutamine tract is sufficient to cause changes in sucrose preference in mice. There was no change in sucrose preference in *Pcp2-Atxn1[82Q]* mice, suggesting that cerebellar cortical dysfunction is not sufficient to cause this effect. We did not observe a statistically significant change in *Atxn1*$^{-/-}$ mice. However, it is difficult to draw conclusions about loss vs gain of function from our *Atxn1*$^{-/-}$ data due to the small sample size and variability caused by leaks and spills during testing. An important next step would be to replicate this data in another group of mice not experiencing the technical difficulties we experienced. If true, the increased sucrose preference in *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ mice could be caused by a number of things, such as an altered ability to taste sucrose, metabolic changes, or changes in motivation. Motivation in particular could be a promising avenue for future research. *ATXN1* helps regulate the levels of the D2 dopamine receptor in the cerebellum (Goold *et al.* 2007). If this is true in the striatum as well, it could provide an explanation for the phenotype we see.

Finally, we used the elevated plus maze to test for anxiety-like phenotypes in our mice. *Atxn1*$^{154Q/2Q}$ mice showed extreme individual variation in open arm time, but their slightly increased closed arm time and greatly decreased center time may suggest an anxiety-like phenotype. The 78Q allele was not sufficient to cause this, as *Atxn1*$^{78Q/2Q}$ mice were no different from wild type. Interestingly, both *Atxn1*$^{-/-}$ and *Pcp2-ATXN1[82Q]* mice showed an *increase* in open arm time relative to wild type, which was especially prominent in the *Pcp2-ATXN1[82Q]* mice. This was the only phenotype we observed that could be caused by expressing mutant *ATXN1* in Purkinje neurons alone,

suggesting that the cerebellum may be involved in approach/avoidance behavior in mice. The abnormal behavior these mice showed during training for the Barnes maze may also be consistent with a reduced fear of brightly lit, exposed spaces. This reduced fear in *Pcp2-ATXN1[82Q]* mice is somewhat reminiscent of the human cerebellar cognitive affective syndrome (CCAS), which leads to impulsiveness and risky behavior (Schmahmann and Sherman 1998), but this is purely speculative at this stage. Any research into CCAS in mice will likely need to rely on a battery of tests and controlled cerebellar lesions.

What could be the cellular mechanism(s) by which *ATXN1* affects behavior? We have begun to investigate this question with a focus on how loss of *ATXN1* might affect learning and memory. The hippocampus is a good candidate region for investigation, as both of the learning and memory tests we used—Barnes maze and context fear conditioning—rely heavily on hippocampal function. Our data show that hippocampal neurogenesis is reduced in *Atxn1*$^{-/-}$ mice (Asher *et al.* 2016). This is also true of *Atxn1*$^{154Q/2Q}$ mice (Cvetanovic, Hu, and Opal 2016).

There are several possible mechanisms for ATXN1's role in neurogenesis. First, ataxin-1 could affect the survival of either stem cells or their progeny. However, we did not observe an increase in cell death in our *Atxn1*$^{-/-}$ neurospheres, as measured by live/dead cell counts and Western blotting for caspase-3 expression (data not shown). This argues against caspase 3-dependent cell death as a major mechanism of reduced neurogenesis but does not exclude the possibility that cell death could occur *in vivo* and/or through caspase 3-independent mechanisms.

Even if it does not affect survival, *ATXN1* could affect the number of available NPCs by affecting their differentiation or self-renewal, as evidenced by the increased degree of differentiation we saw in *Atxn1*$^{-/-}$ neurospheres. It is worth noting that ataxin-1 suppresses the Notch signaling pathway in *Drosophila* (Tong *et al*. 2011), which regulates maintenance of neural stem cells (Giachino and Taylor 2014). *ATXN1* also regulates expression of *Tgfβ* (Crespo-Barreto *et al*. 2010), which is a critical regulator of NPC differentiation (Chen *et al*. 2013; Tapia-González *et al.* 2013; Daynac *et al.* 2014).

Finally, *ATXN1* could be involved in controlling cell cycle entry or progression, thus directly affecting the frequency or rate of cell divisions. There is a growing body of literature showing that *ATXN1* could affect the cell cycle at multiple points. First, the ataxin-1 protein physically interacts with several regulators of cell cycle progression (Hosp *et al.* 2015). These include Cdc16 and Bub3, which are components of the spindle assembly checkpoint at the metaphase/anaphase transition (Passmore *et al*. 2005; Han *et al*. 2014; Huang and Bonni 2016); Cdk1, which among other things helps to control entry into mitosis and later into anaphase (Gavet *et al*. 2010; Fujimitsu, Grimaldi, and Yamano 2016); and Cdk2, which functions at the G1/S phase transition (Malumbres and Barbacid 2005). The G1/S phase transition in particular is also regulated by other proteins which may be under the control of *ATXN1*. In flies, *ATXN1*'s binding partner Capicua controls expression of Cyclin E, the cyclin which associates with Cdk2 during the G1/S phase transition (Krivy, Bradley-Gill, and Moon 2013). In addition, *ATXN1* regulates transcription of Cyclin D1 (Crespo-Barreto *et al*. 2010), a major regulator of progression through G0/G1 into S phase (Baldin *et al.* 1993). Interestingly, we have found that

Cyclin D1 levels are reduced in hippocampal NPCs from *Atxn1*$^{-/-}$ mice, and that the percentage of cells in the G0/G1 phase is increased at the expense of S phase (Asher *et al*. 2016). This may indicate that *ATXN1* affects entry into the cell cycle via Cyclin D1, Cyclin E, or Cdk2. However, further work is needed to determine whether the reduced Cyclin D1 levels are a cause or an effect of the *ATXN1*-mediated decrease in proliferation, and whether Cyclin E and Cdk2 are affected in NPCs.

Neurogenesis makes important contributions to hippocampal plasticity and learning (Christian, Song, and Ming 2014), so the deficit in neurogenesis could contribute to the behavioral phenotypes we have found in *Atxn1*$^{-/-}$ and *Atxn1*$^{154Q/2Q}$ mice. However, it is likely not the only way that *ATXN1* affects hippocampal function. Indeed, previous studies have shown reduced hippocampal dendritic arborization in *Atxn1*$^{154Q/2Q}$ mice (Watase *et al*. 2007) and deficits in hippocampal paired-pulse facilitation in *Atxn1*$^{-/-}$ mice (Matilla *et al.* 1998). Beyond the hippocampus, ataxin-1 and its binding partner Capicua also affect cortical thickness (Lu *et al*. 2017). In the future, it will be important both to determine the relative contributions of each of these processes to ataxin-1's effect on behavior *in vivo* and to elucidate the molecular mechanisms for further study and/or treatment. This effort would benefit greatly from an *ATXN1* flox mouse line, which does not yet exist but would make it much easier to test *ATXN1* function in specific regions through conditional deletion.

This thesis research helps to establish cognition, mood, and the regulation of adult hippocampal neurogenesis as important endogenous functions of *ATXN1*. Future basic science research can delve further into the molecular mechanisms of these effects,

helping to fill out our knowledge of the many processes controlled by *ATXN1*. From a clinical standpoint, this research is relevant both to SCA1, in which patients may experience cognitive deficits, and to other diseases including Alzheimer's disease, where *ATXN1* may contribute to risk (Bertram *et al.* 2008). Future translational research can focus on improving cognition in these diseases and avoiding cognitive side effects of treatments which manipulate *ATXN1*.

References

Asher, Melissa, Andrea Johnson, Bojana Zecevic, David Pease, and Marija Cvetanovic. 2016. "Ataxin-1 Regulates Proliferation of Hippocampal Neural Precursors." *Neuroscience* 322. IBRO: 54–65.

Baldin, V., J. Lukas, M. J. Marcote, M. Pagano, and G. Draetta. 1993. "Cyclin D1 Is a Nuclear Protein Required for Cell Cycle Progression in G1." *Genes & Development* 7 (5): 812–21.

Banfi, S., A. Servadio, M. Chung, F. Capozzoli, L. a. Duvick, R. Elde, H. Y. Zoghbi, and H. T. Orr. 1996. "Cloning and Developmental Expression Analysis of the Murine Homolog of the Spinocerebellar Ataxia Type 1 Gene (Sca1)." *Human Molecular Genetics* 5 (1): 33–40.

Barnes, C. A. 1979. "Memory Deficits Associated With Senescence: A Neurophysiological and Behavioral Study in the Rat." *Journal of Comparative and Physiological Psychology* 93 (1): 74–104.

Bertram, Lars, Christoph Lange, Kristina Mullin, Michele Parkinson, Monica Hsiao, Meghan F. Hogan, Brit M. M. Schjeide, *et al*. 2008. "Genome-Wide Association Analysis Reveals Putative Alzheimer's Disease Susceptibility Loci in Addition to APOE." *American Journal of Human Genetics* 83 (5): 623–32.

Bodranghien, Florian, Amy Bastian, Carlo Casali, Mark Hallett, Elan D. Louis, Mario Manto, Peter Mariën, *et al*. 2016. "Consensus Paper: Revisiting the Symptoms and Signs of Cerebellar Syndrome." *Cerebellum* 15 (3). Springer US: 369–91.

Bonaguidi, Michael A., Chian-Yu Peng, Tammy McGuire, Gustave Falciglia, Kevin T. Gobeske, Catherine Czeisler, and John A. Kessler. 2008. "Noggin Expands Neural Stem Cells in the Adult Hippocampus." *Journal of Neuroscience* 28 (37).

Bonaguidi, Michael A., Michael A. Wheeler, Jason S. Shapiro, Ryan P. Stadel, Gerald J. Sun, Guo-Li Ming, and Hongjun Song. 2011. "*In Vivo* Clonal Analysis Reveals Self-Renewing and Multipotent Adult Neural Stem Cell Characteristics." *Cell* 145 (7): 1142–55.

Braun, Simon M. G., and Sebastian Jessberger. 2014. "Adult Neurogenesis: Mechanisms and Functional Significance." *Development* 141 (10): 1983–86.

Buckner, Randy L., Fenna M. Krienen, Angela Castellanos, Julio C. Diaz, and B. T. Thomas Yeo. 2011. "The Organization of the Human Cerebellum Estimated by Intrinsic Functional Connectivity." *Journal of Neurophysiology* 106 (5): 2322–45.

Bürk, K., C. Globas, S. Bösch, T. Klockgether, C. Zühlke, I. Daum, and J. Dichgans. 2003. "Cognitive Deficits in Spinocerebellar Ataxia Type 1, 2, and 3." *Journal of Neurology* 250 (2): 207–11.

Burright, E. N., H. B. Clark, A. Servadio, T. Matilla, R. M. Feddersen, W. S. Yunis, L. A. Duvick, H. Y. Zoghbi, and H. T. Orr. 1995. "SCA1 Transgenic Mice: A Model for Neurodegeneration Caused by an Expanded CAG Trinucleotide Repeat." *Cell* 82 (6): 937–48.

Celestino-Soper, Patrícia B. S., Cindy Skinner, Richard Schroer, Patricia Eng, Jayant Shenai, Malgorzata M. J. Nowaczyk, Deborah Terespolsky, *et al*. 2012. "Deletions in Chromosome 6p22.3-p24.3, Including ATXN1, Are Associated with Developmental Delay and Autism Spectrum Disorders." *Molecular Cytogenetics*. BioMed Central Ltd. doi:10.1186/1755-8166-5-17.

Chen, Hung-Kai, Pedro Fernandez-Funez, Summer F. Acevedo, Yung C. Lam, Michael D. Kaytor, Michael H. Fernandez, Alastair Aitken, *et al*. 2003. "Interaction of Akt-Phosphorylated Ataxin-1 with 14-3-3 Mediates Neurodegeneration in Spinocerebellar Ataxia Type 1." *Cell* 113 (4): 457–68.

Chen, Jia-Yun, Jia-Ren Lin, Feng-Chiao Tsai, and Tobias Meyer. 2013. "Dosage of Dyrk1a Shifts Cells within a p21-Cyclin D1 Signaling Map to Control the Decision to Enter the Cell Cycle." *Molecular Cell* 52 (1): 87–100.

Christian, Kimberly M., Hongjun Song, and Guo-Li Ming. 2014. "Functions and Dysfunctions of Adult Hippocampal Neurogenesis." *Annual Review of Neuroscience*, no. May: 243–62.

Commons, Kathryn G., Aram B. Cholanians, Jessica A. Babb, and Daniel G. Ehlinger. 2017. "The Rodent Forced Swim Test Measures Stress-Coping Strategy, Not Depression-like Behavior." *ACS Chemical Neuroscience* 8 (5): 955–60.

Crespo-Barreto, Juan, John D. Fryer, Chad A. Shaw, Harry T. Orr, and Huda Y. Zoghbi. 2010. "Partial Loss of Ataxin-1 Function Contributes to Transcriptional Dysregulation in Spinocerebellar Ataxia Type 1 Pathogenesis." *PLoS Genetics* 6 (7). Public Library of Science: e1001021.

Cvetanovic, Marija, Yuan-Shih Hu, and Puneet Opal. 2016. "Mutant Ataxin-1 Inhibits Neural Progenitor Cell Proliferation in SCA1." *Cerebellum*, June. Springer US, 1–8.

Daynac, Mathieu, Jose R. Pineda, Alexandra Chicheportiche, Laurent R. Gauthier, Lise Morizur, François D. Boussin, and Marc-André Mouthon. 2014. "TGFβ Lengthens the G1 Phase of Stem Cells in Aged Mouse Brain." *Stem Cells* 32 (12): 3257–65.

Deng, Wei, Michael D. Saxe, Iryna S. Gallina, and Fred H. Gage. 2009. "Adult-Born Hippocampal Dentate Granule Cells Undergoing Maturation Modulate Learning and Memory in the Brain." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 29 (43): 13532–42.

Doetsch, Fiona, and Rene Hen. 2005. "Young and Excitable: The Function of New Neurons in the Adult Mammalian Brain." *Current Opinion in Neurobiology* 15 (1): 121–28.

Dupret, David, Jean-Michel Revest, Muriel Koehl, François Ichas, Francesca De Giorgi, Pierre Costet, Djoher Nora Abrous, and Pier Vincenzo Piazza. 2008. "Spatial Relational Memory Requires Hippocampal Adult Neurogenesis." *PloS One* 3 (4): e1959.

E Keren-Happuch, Shen-Hsing Annabel Chen, Moon-Ho Ringo Ho, and John E. Desmond. 2014. "A Meta-Analysis of Cerebellar Contributions to Higher Cognition from PET and fMRI Studies." *Human Brain Mapping* 35 (2). NIH Public Access: 593–615.

Faigle, Roland, and Hongjun Song. 2013. "Signaling Mechanisms Regulating Adult Neural Stem Cells and Neurogenesis." *Biochimica et Biophysica Acta* 1830 (2): 2435–48.

Fedele, V., L. Roybon, U. Nordström, J. Y. Li, and P. Brundin. 2011. "Neurogenesis in the R6/2 Mouse Model of Huntington's Disease Is Impaired at the Level of NeuroD1." *Neuroscience* 173 (January): 76–81.

Fujimitsu, K., M. Grimaldi, and H. Yamano. 2016. "Cyclin-Dependent Kinase 1-Dependent Activation of APC/C Ubiquitin Ligase." *Science* 352 (6289): 1121–24.

Gavet, Olivier, Jonathon Pines, A. Abrieu, T. Brassac, S. Galas, D. Fisher, J. C. Labbé, *et al*. 2010. "Progressive Activation of CyclinB1-Cdk1 Coordinates Entry to Mitosis." *Developmental Cell* 18 (4). Elsevier: 533–43.

Giachino, Claudio, and Verdon Taylor. 2014. "Notching up Neural Stem Cell Homogeneity in Homeostasis and Disease." *Frontiers in Neuroscience* 8 (January). Frontiers: 32.

Goold, Robert, Michael Hubank, Abigail Hunt, Janice Holton, Rajesh P. Menon, Tamas Revesz, Massimo Pandolfo, and Antoni Matilla-Dueñas. 2007. "Down-Regulation of the Dopamine Receptor D2 in Mice Lacking Ataxin 1." *Human Molecular Genetics* 16 (17): 2122–34.

Guo, Christine C., Rachel Tan, John R. Hodges, Xintao Hu, Saber Sami, and Michael Hornberger. 2016. "Network-Selective Vulnerability of the Human Cerebellum to Alzheimer's Disease and Frontotemporal Dementia." *Brain: A Journal of Neurology* 139 (5).

Handley, S. L., and S. Mithani. 1984. “Effects of Alpha-Adrenoceptor Agonists and Antagonists in a Maze-Exploration Model of ‘Fear’-Motivated Behaviour.” *Naunyn-Schmiedeberg’s Archives of Pharmacology* 327 (1): 1–5.

Han, Joo Seok, Benjamin Vitre, Daniele Fachinetti, and Don W. Cleveland. 2014. “Bimodal Activation of BubR1 by Bub3 Sustains Mitotic Checkpoint Signaling.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (40). National Academy of Sciences: E4185–93.

Harrison, F. E., A. H. Hosseini, and M. P. McDonald. 2009. “Endogenous Anxiety and Stress Responses in Water Maze and Barnes Maze Spatial Memory Tasks.” *Behavioural Brain Research* 198 (1). NIH Public Access: 247–51.

Hodge, Rebecca D., Branden R. Nelson, Robert J. Kahoud, Roderick Yang, Kristin E. Mussar, Steven L. Reiner, and Robert F. Hevner. 2012. “Tbr2 Is Essential for Hippocampal Lineage Progression from Neural Stem Cells to Intermediate Progenitors and Neurons.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 32 (18): 6275–87.

Hosp, Fabian, Hannes Vossfeldt, Matthias Heinig, Djordje Vasiljevic, Anup Arumughan, Emanuel Wyler, Markus Landthaler, *et al*. 2015. “Quantitative Interaction Proteomics of Neurodegenerative Disease Proteins.” *Cell Reports* 11 (7). The Authors: 1134–46.

Huang, Ju, and Azad Bonni. 2016. “A Decade of the Anaphase-Promoting Complex in the Nervous System.” *Genes & Development* 30 (6). Cold Spring Harbor Laboratory Press: 622–38.

Illouz, Tomer, Ravit Madar, Charlotte Clague, Kathleen J. Griffioen, Yoram Louzoun, and Eitan Okun. 2016. “Unbiased Classification of Spatial Strategies in the Barnes Maze.” *Bioinformatics* 32 (21). Oxford University Press: 3314–20.

Iñiguez, Sergio D., Brandon L. Warren, and Carlos A. Bolaños-Guzmán. 2010. “Short- and Long-Term Functional Consequences of Fluoxetine Exposure during Adolescence in Male Rats.” *Biological Psychiatry* 67 (11): 1057–66.

Jessberger, Sebastian, Robert E. Clark, Nicola J. Broadbent, Gregory D. Clemenson Jr, Antonella Consiglio, D. Chichung Lie, Larry R. Squire, and Fred H. Gage. 2009. “Dentate Gyrus-Specific Knockdown of Adult Neurogenesis Impairs Spatial and Object Recognition Memory in Adult Rats.” *Learning & Memory* 16 (2): 147–54.

Johnston, Stephen T., Matthew Shtrahman, Sarah Parylak, J. Tiago Gonçalves, and Fred H. Gage. 2015. “Paradox of Pattern Separation and Adult Neurogenesis: A Dual Role for New Neurons Balancing Memory Resolution and Robustness.” *Neurobiology of Learning and Memory*, November. doi:10.1016/j.nlm.2015.10.013.

Kish, S. J., M. el-Awar, L. Schut, L. Leach, M. Oscar-Berman, and M. Freedman. 1988. “Cognitive Deficits in Olivopontocerebellar Atrophy: Implications for the Cholinergic Hypothesis of Alzheimer’s Dementia.” *Annals of Neurology* 24 (2): 200–206.

Klinke, Ina, Martina Minnerop, Tanja Schmitz-Hübsch, Marc Hendriks, Thomas Klockgether, Ullrich Wüllner, and Christoph Helmstaedter. 2010. “Neuropsychological Features of Patients with Spinocerebellar Ataxia (SCA) Types 1, 2, 3, and 6.” *Cerebellum* 9 (3): 433–42.

Ko, Hyoung-Gon, Deok-Jin Jang, Junehee Son, Chuljung Kwak, Jun-Hyeok Choi, Young-Hoon Ji, Yun-Sil Lee, Hyeon Son, and Bong-Kiun Kaang. 2009. “Effect of Ablated Hippocampal Neurogenesis on the Formation and Extinction of Contextual Fear Memory.” *Molecular Brain* 2 (January): 1.

Koziol, Leonard F., Deborah Budding, Nancy Andreasen, Stefano D’Arrigo, Sara Bulgheroni, Hiroshi Imamizu, Masao Ito, *et al*. 2014. “Consensus Paper: The Cerebellum’s Role in Movement and Cognition.” *Cerebellum* 13 (1): 151–77.

Krivy, Kate, Mary-Rose Bradley-Gill, and Nam-Sung Moon. 2013. “Capicua Regulates Proliferation and Survival of RB-Deficient Cells in Drosophila.” *Biology Open* 2 (2): 183-90.

Lam, Yung C., Aaron B. Bowman, Paymaan Jafar-Nejad, Janghoo Lim, Ronald Richman, John D. Fryer, Eric D. Hyun, *et al*. 2006. “ATAXIN-1 Interacts with the Repressor Capicua in Its Native Complex to Cause SCA1 Neuropathology.” *Cell* 127 (7): 1335–47.

Lim, Janghoo, Juan Crespo-Barreto, Paymaan Jafar-Nejad, Aaron B. Bowman, Ronald Richman, David E. Hill, Harry T. Orr, and Huda Y. Zoghbi. 2008. “Opposing Effects of Polyglutamine Expansion on Native Protein Complexes Contribute to SCA1.” *Nature* 452 (7188): 713–18.

Lo, Raymond Y., Karla P. Figueroa, Stefan M. Pulst, Susan Perlman, George Wilmot, Christopher Gomez, Jeremy Schmahmann, *et al*. 2016. “Depression and Clinical Progression in Spinocerebellar Ataxias.” *Parkinsonism & Related Disorders* 22 (January): 87–92.

Lorenzetti, D., K. Watase, B. Xu, M. M. Matzuk, H. T. Orr, and H. Y. Zoghbi. 2000. “Repeat Instability and Motor Incoordination in Mice with a Targeted Expanded CAG Repeat in the Sca1 Locus.” *Human Molecular Genetics* 9 (5). Oxford University Press: 779–85.

Lu, Hsiang-Chih, Qiumin Tan, Maxime W. C Rousseaux, Wei Wang, Ji-Yoen Kim, Ronald Richman, Ying-Wooi Wan, *et al*. 2017. “Disruption of the ATXN1–CIC Complex Causes a Spectrum of Neurobehavioral Phenotypes in Mice and Humans.” *Nature Genetics*. doi:10.1038/ng.3808.

Ma, Dengke K., Woon Ryoung Kim, Guo-Li Ming, and Hongjun Song. 2009. “Activity-Dependent Extrinsic Regulation of Adult Olfactory Bulb and Hippocampal Neurogenesis.” *Annals of the New York Academy of Sciences* 1170 (1). Wiley Online Library: 664–73.

Malumbres, Marcos, and Mariano Barbacid. 2005. “Mammalian Cyclin-Dependent Kinases.” *Trends in Biochemical Sciences* 30 (11): 630–41.

Matilla, A., E. D. Roberson, S. Banfi, J. Morales, D. L. Armstrong, E. N. Burright, H. T. Orr, J. D. Sweatt, H. Y. Zoghbi, and M. M. Matzuk. 1998. “Mice Lacking Ataxin-1 Display Learning Deficits and Decreased Hippocampal Paired-Pulse Facilitation.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 18 (14): 5508–16.

Matilla, T., V. Volpinl, D. Genis, J. Rosell, J. Corral, A. Davalos, A. Molins, and X. Estivill. 1993. “Presymptomatic Analysis of Spinocerebellar Ataxia Type 1 (SCA1) via the Expansion of the SCA1 CAG-Repeat in a Large Pedigree Displaying Anticipation and Parental Male Bias.” *Human Molecular Genetics* 2 (12). Oxford University Press: 2123–28.

Moriarty, Amy, Arron Cook, Helen Hunt, Matthew E. Adams, Lisa Cipolotti, and Paola Giunti. 2016. “A Longitudinal Investigation into Cognition and Disease Progression in Spinocerebellar Ataxia Types 1, 2, 3, 6, and 7.” *Orphanet Journal of Rare Diseases* 11 (1). BioMed Central: 82.

Nakashiba, Toshiaki, Jesse D. Cushman, Kenneth A. Pelkey, Sophie Renaudineau, Derek L. Buhl, Thomas J. McHugh, Vanessa Rodriguez Barrera, *et al*. 2012. “Young Dentate Granule Cells Mediate Pattern Separation, Whereas Old Granule Cells Facilitate Pattern Completion.” *Cell* 149 (1): 188–201.

O’Keefe, J., J. Dostrovsky - Brain research, and 1971. 1971. “The Hippocampus as a Spatial Map. Preliminary Evidence from Unit Activity in the Freely-Moving Rat.” *Elsevier Oceanography Series*.

O’Leary, Timothy P., and Richard E. Brown. 2013. “Optimization of Apparatus Design and Behavioral Measures for the Assessment of Visuo-Spatial Learning and Memory of Mice on the Barnes Maze.” *Learning & Memory* 20 (2). Cold Spring Harbor Laboratory Press: 85–96.

Orr, H. T., M. Y. Chung, S. Banfi, T. J. Kwiatkowski, A. Servadio, a. L. Beaudet, a. E. McCall, L. a. Duvick, L. P. Ranum, and H. Y. Zoghbi. 1993. "Expansion of an Unstable Trinucleotide CAG Repeat in Spinocerebellar Ataxia Type 1." *Nature Genetics* 4 (3): 221–26.

Passmore, Lori A., Christopher R. Booth, Catherine Vénien-Bryan, Steven J. Ludtke, Céline Fioretto, Louise N. Johnson, Wah Chiu, and David Barford. 2005. "Structural Analysis of the Anaphase-Promoting Complex Reveals Multiple Active Sites and Insights into Polyubiquitylation." *Molecular Cell* 20 (6): 855–66.

Porsolt, R. D., A. Bertin, and M. Jalfre. 1977. "Behavioral Despair in Mice: A Primary Screening Test for Antidepressants." *Archives Internationales de Pharmacodynamie et de Therapie* 229 (2): 327–36.

Quan, F., J. Janas, and B. W. Popovich. 1995. "A Novel CAG Repeat Configuration in the SCA1 Gene: Implications for the Molecular Diagnostics of Spinocerebellar Ataxia Type 1." *Human Molecular Genetics* 4 (12): 2411–13.

Sahay, Amar, Kimberly N. Scobie, Alexis S. Hill, Colin M. O'Carroll, Mazen A. Kheirbek, Nesha S. Burghardt, André A. Fenton, Alex Dranovsky, and René Hen. 2011. "Increasing Adult Hippocampal Neurogenesis Is Sufficient to Improve Pattern Separation." *Nature* 472 (7344): 466–70.

Schmahmann, Jeremy D., and Janet C. Sherman. 1998. "The Cerebellar Cognitive Affective Syndrome." *Brain: A Journal of Neurology* 121 (4): 561–79.

Schmitz-Hübsch, Tanja, Mathieu Coudert, Sophie Tezenas du Montcel, Paola Giunti, Robyn Labrum, Alexandra Dürr, Pascale Ribai, *et al*. 2011. "Depression Comorbidity in Spinocerebellar Ataxia." *Movement Disorders: Official Journal of the Movement Disorder Society* 26 (5): 870–76.

Scoville, William Beecher, and Brenda Milner. 1957. "Loss of Recent Memory after Bilateral Hippocampal Lesions." *Journal of Neurology, Neurosurgery, and Psychiatry* 20 (1). BMJ Group: 11.

Servadio, Antonio, Beena Koshy, Dawna Armstrong, Barbara Antalffy, Harry T. Orr, and Huda Y. Zoghbi. 1995. "Expression Analysis of the ataxin–1 Protein in Tissues from Normal and Spinocerebellar Ataxia Type 1 Individuals." *Nature Genetics* 10 (1). Nature Publishing Group: 94–98.

Shima, T., K. Inada, M. Ito, S. Saito, R. Bulla, F. Tedesco, S. Scherjon, *et al*. 2013. "Creating a False Memory in the Hippocampus." *Science* 341 (July): 387–91.

Shors, T. J., G. Miesegaes, A. Beylin, M. Zhao, T. Rydel, and E. Gould. 2001. "Neurogenesis in the Adult Is Involved in the Formation of Trace Memories." *Nature* 410 (6826): 372–76.

Song, Juan, Chun Zhong, Michael A. Bonaguidi, Gerald J. Sun, Derek Hsu, Yan Gu, Konstantinos Meletis, *et al*. 2012. "Neuronal Circuitry Mechanism Regulating Adult Quiescent Neural Stem-Cell Fate Decision." *Nature* 489 (7414): 150–54.

Stoodley, Catherine J., Jason P. MacMore, Nikos Makris, Janet C. Sherman, and Jeremy D. Schmahmann. 2016. "Location of Lesion Determines Motor vs. Cognitive Consequences in Patients with Cerebellar Stroke." *NeuroImage: Clinical* 12: 765–75.

Stoodley, Catherine J., and Jeremy D. Schmahmann. 2009. "Functional Topography in the Human Cerebellum: A Meta-Analysis of Neuroimaging Studies." *NeuroImage* 44 (2): 489–501.

Stoodley, Catherine J., and Jeremy D. Schmahmann. 2010. "Evidence for Topographic Organization in the Cerebellum of Motor Control versus Cognitive and Affective Processing." *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior* 46 (7). NIH Public Access: 831–44.

Tapia-González, Silvia, M. Dolores Muñoz, M. Isabel Cuartero, and Amelia Sánchez-Capelo. 2013. "Smad3 Is Required for the Survival of Proliferative Intermediate Progenitor Cells in the Dentate Gyrus of Adult Mice." *Cell Communication and Signaling: CCS* 11 (December): 93.

Tedesco, Anna M., Francesca R. Chiricozzi, Silvia Clausi, Michela Lupo, Marco Molinari, and Maria G. Leggio. 2011. "The Cerebellar Cognitive Profile." *Brain: A Journal of Neurology* 134 (12).

Tong, Xin, Hongxing Gui, Feng Jin, Bryan W. Heck, Peihui Lin, Jianjie Ma, Joseph D. Fondell, and Chih-Cheng Tsai. 2011. "Ataxin-1 and Brother of Ataxin-1 Are Components of the Notch Signalling Pathway." *EMBO Reports* 12 (5). Nature Publishing Group: 428–35.

Verret, Laure, Joanna L. Jankowsky, Guilian M. Xu, David R. Borchelt, and Claire Rampon. 2007. "Alzheimer's-Type Amyloidosis in Transgenic Mice Impairs Survival of Newborn Neurons Derived from Adult Hippocampal Neurogenesis." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27 (25): 6771–80.

Watase, Kei, Jennifer R. Gatchel, Yaling Sun, Effat Emamian, Richard Atkinson, Ronald Richman, Hidehiro Mizusawa, *et al*. 2007. "Lithium Therapy Improves Neurological Function and Hippocampal Dendritic Arborization in a Spinocerebellar Ataxia Type 1 Mouse Model." Edited by Steven E. Hyman. *PLoS Medicine* 4 (5). Public Library of Science: e182.

Watase, Kei, Edwin J. Weeber, Bisong Xu, Barbara Antalffy, Lisa Yuva-Paylor, Kouichi Hashimoto, Masanobu Kano, *et al*. 2002. "A Long CAG Repeat in the Mouse Sca1 Locus Replicates SCA1 Features and Reveals the Impact of Protein Solubility on Selective Neurodegeneration." *Neuron* 34 (6): 905–19.

Willner, P., A. Towell, D. Sampson, S. Sophokleous, and R. Muscat. 1987. "Reduction of Sucrose Preference by Chronic Unpredictable Mild Stress, and Its Restoration by a Tricyclic Antidepressant." *Psychopharmacology* 93: 358–64.

Winner, Beate, D. Chichung Lie, Edward Rockenstein, Robert Aigner, Ludwig Aigner, Eliezer Masliah, H. Georg Kuhn, and Jürgen Winkler. 2004. "Human Wild-Type α-Synuclein Impairs Neurogenesis." *Journal of Neuropathology and Experimental Neurology* 63 (11). Oxford University Press: 1155–66.

Zoghbi, H. Y., M. S. Pollack, L. A. Lyons, R. E. Ferrell, S. P. Daiger, and A. L. Beaudet. 1988. "Spinocerebellar Ataxia: Variable Age of Onset and Linkage to Human Leukocyte Antigen in a Large Kindred." *Annals of Neurology* 23 (6): 580–84.

Zu, Tao, Lisa A. Duvick, Michael D. Kaytor, Michael S. Berlinger, Huda Y. Zoghbi, H. Brent Clark, and Harry T. Orr. 2004. "Recovery from Polyglutamine-Induced Neurodegeneration in Conditional SCA1 Transgenic Mice." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 24 (40): 8853–61.